PLAGUE

Conquest and Eradication in India

S.C. SEAL

INDIAN COUNCIL OF MEDICAL RESEARCH
NEW DELHI
1987

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47/1, (First Floor) St. Marks Road,
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Printed at Gidson Printing Works,
FS-5, Tagore Garden, New Delhi 110027

Preface

Plague is one of the ancient, dreadful and most formidable pestilential diseases that ravaged the world for many centuries and caused millions of deaths several times in a large number of countries of the world, especially Europe, Central Asia, China, Africa, South America, North America, India and other parts of South-East Asia. Although work in the field of natural sciences was carried out assiduously all the time depending on the existing knowledge, beliefs, and ideas of the time, it was in India that the solution of the problem came in sight for the first time after the discovery of plague bacillus by Yersin and Kitasato in 1894, and through extensive field observations and laboratory research on bacteriology and epidemiology of the disease by various workers like Simond, Ogata, Haffkine, Bacot and several National and International Plague Commissions, particularly the Indian Plague Commission, during the first decade of the present century.

The discovery of Plague vaccine by Haffkine at the Bombay Bacteriological Laboratory can be reckoned as the earliest landmark of introduction of a fairly effective prophylactic measure against plague. Effective drugs for the treatment of the disease were found only 45 years ago with the discovery of sulpha drugs followed by antibiotics like streptomycin and several others. Prior to this, the Haffkine Institute had started production of anti-plague horse serum which though fairly effective in the treatment of plague, needed improvement and standardisation of the methods of production and estimation of its antibody titre, for which the in-bred white mouse was found suitable at the Haffkine Institute.

The utilisation of DDT as one of the most effective insecticides during the Second World War in the forties of this century is another landmark in the armoury of control of plague. In other words, measures for control, treatment and prevention of plague are a little over four decades old. But once a complete knowledge of the epidemiology of the disease, its causative germ with clear definitions of its morphological, sero-immunological and chemical antigenic structure, mode of transmission, reservoirs and host behaviour were obtained by the present author the problem became fully amenable to control and even eradication.

The author, however, noted in the early forties of this century that in spite of the fair knowledge available on the rodent reservoirs, vector fleas, mode of transmission and clinical features, knowledge about bacteriology,

antigenic structure, serology, immunisation, production of vaccines and anti-sera and even treatment was still very vague and incomplete. The most important handicap in this respect was the identification of the characteristics of the organism which being autoagglutinable when cultured in the available media, could not be effectively used for identification, serology, immunology, and for the isolation of a purified antigen. In all these aspects the present author has been able to contribute a better and more complete picture of the cultural, morphological, antigenic, serological and immunological characters followed by its ecological and epidemiological aspects through field as well as experimental epidemiological studies including surveillance operations, immunology, treatment, prevention and control.

The latest publication in English language dealing comprehensively with the plague problems available to the author when he undertook the work in the early forties was the manual published in 1936 by Wu-Lien-Teh, Chun, Pollitzer and Wu and the advances made by Rowland, Sokhey, Girard, Meyer, Schutze, Devignat, Otten, Macchiavello, Korobkova and other Russian workers. The latest excellent treatise on the subject was, however, that of Pollitzer published under the auspices of the World Health Organisation in 1954.

The work carried out by the author at the Haffkine Institute, Bombay, between 1940 and 1943 clarified most of the difficulties encountered earlier. In fact the discovery of the protein-free casein hydrolysate, liquid and solid media with enrichment completely revolutionised the idea about the characteristics of the organism itself. It not only permitted isolation of specific plague antigen in pure form but also facilitated studies on morphological, serological and immunological characters, production of better vaccine and antiserum as well as various epidemiological works including surveillance and control, and finally eradication. His field and extended experimental work has clarified all the epidemic phases, *e.g.*, pre-, epidemic, post and inter-epidemic, through follow up work for 28 years in Calcutta between 1948 and 1976. He had also opportunites of investigating certain epidemics in Assam, West Bengal, Bihar, Madras and Mysore that led to the complete cessation of plague cases in India since 1968, making eradication possible in other countries as well. This compendium is an attempt to consolidate all his findings in the background of the chronological development of knowledge on the subject beginning from the primitive stage to the modern period. In fact, it is only when the subject is viewed from the historical standpoint that we can realize the magnitude of the debt to the pioneers of the past and the task still remaining to be accomplished.

Finally, the author wishes to suggest that the present compilation is expected to be a good example of a complete ecological study of a disease beginning with a mysterious origin to the complete solution of the problem, the necessary knowledge being acquired step by step with meticulous care and perseverance commensurate with the existing knowledge at the particular period of time. This compilation is prepared with the idea that not only

the health authorities of the affected countries but also the research students of epidemiology can draw inspiration from the complete work as an appropriate and useful model of an epidemiological study and a practical exercise from the learner's point of view.

The entire historical record and chronological order of development of knowledge about plague have been divided broadly into five periods *viz.*, (1) primitive way of thinking and practices such as, positive animism, magic, black art, sorcery *etc*; (2) idea developing about natural causes of disease beginning from Hippocrates; (3) the beginning of modern research, theory of contagion, role of reservoirs (rodents) in the transmission of plague *etc.*, till the discovery of the causative germ by Yersin and Kitasa to in 1894; (4) period beginning with the discovery of the plague bacillus up to 1940 during which studies related to causation of epidemics and pandemics, control of sources and vectors and some studies in bacteriology, immunology, treatment, control and prevention were carried out; and (5) the last phase in the development of knowledge about the disease beginning from the forties to date (1982).

The author's work covers mainly the last phase providing fairly detailed analysis of the natural phenomena including the findings of his own which have played a distinct and substantial role in the conquest of the disease in India with the hope that such results can be achieved by those countries which still report human plague cases.

March, 1987
Calcutta

S.C. Seal

Acknowledgment

In the above task the author is indebted to the Indian Council of Medical Research (formerly Indian Research Fund Association) for the financial support during the earlier part of his work and also between 1970 and 1973 as Emeritus Medical Scientist. He is thankful to the Late Major General Sir Sahib Singh Sokhey, Ex-Director, Haffkine Institute, Bombay' for his encouragement and support while working at that Institute between 1940 and 1943.

Thanks are also due to the Health Department of the Government of West Bengal and the Calcutta Corporation and to the Indian Jute Mills Association for their generous help during the management of the plague epidemic in Calcutta between 1948 and 1952.

I am grateful to the Director of the All-India Institute of Hygiene and Public Health, Calcutta, for providing continuous facilities for both field and experimental epidemiological work at the Institute.

His appreciation goes to the entire group of doctors who worked in this project as associates in one or more investigative work, namely, Late Drs K.C. Patnaik and P.N. Bose, Dr L.M. Bhattacharji, Dr A.L. Saha, Major B. Mukherji and Shri S.P. Mukherji (Chemist) and various junior field staff engaged in vector and rat control work.

Special mention may be made of Dr K.K. Modak, Controller of Plague Control Laboratory of the Government of West Bengal, and of Shri R.N. Banerjee, Rodent Control Officer of the surveillance work of this unit and for their ready and spontaneous cooperation in the field of epidemiological work.

The author also wishes to extend his appreciation of the technical assistance given ungrudgingly by Shriyuts Shivapada Mukherjee and K.N. Ghosh, the two Laboratory assistants attached to the Plague Research Unit under the author.

The author is also grateful to the Indian Council of Medical Research, New Delhi, particularly the former Director-General, Prof. V. Ramalingaswami and the present Director-General, Prof. A.S. Paintal, for agreeing to publish this book, and the staff of the Division of Publication & Information, ICMR, New Delhi, for having edited and published this publication.

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CHAPTER I

Introduction

Plague which was one of the most dreaded diseases that haunted the world for centuries with its epidemic, pandemic and endemic ravages and had killed millions of people, is on the way to recede from the world. This is the result of continuous search for and acquiring knowledge about the various ecological processes *viz.*, natural history, etiology, reservoirs, transmission and spread, host reactions and other epidemiological behaviours leading to the discovery of methods of treatment, surveillance, control, prevention and finally to its possible eradication. In fact, this study will serve as an ideal example of an epidemiological model of a disease in which the final conquest was attained by studying the disease on thorough epidemiological principles and methods and thereby acquiring knowledge by stages till the various aspects of the disease processes gradually unfurled themselves and permitted development of appropriate remedies leading to the vanishing point in India by the year 1968. Almost the entire knowledge of plague as it stands today has been acquired during the first half of the present century, in which the workers from India have contributed the larger share.

The purpose of this book is broadly to describe the present status of this knowledge and the relationship its bears to the ultimate conquest of this great pestilence of the world with particular reference to the position of the disease in India and other countries.

Historical Summary of Epidemics of Plague

From the evolutionary point of view man acquired most of the diseases from the animal kingdom by contact or through vectors and plague is only one of them which man even now gets from animals, particularly the rodents. Only under special circumstances was the infection transmitted from man to man when the organisms aberrated into a special virulent from causing pneumonic plague and serious havoc among human beings. But in doing so it underwent the risk of complete destruction with cessation of epidemic. It could only reappear from infected rodents or other animal reservoirs.

The first human epidemic on record according to Wu-Lien-Teh *et al* (1936) was the outbreak among the Philistines in 1320 BC. It was characterised by the appearance of "emerods in their secret parts" as described in

the Bible (I. Samuel, v and vi). The Indian scriptures like the *Bhagvata Purana* (1500-600 BC), however, gives the disease an earlier antiquity by referring to the deaths caused by an epidemic disease preceded by an epizootic among rats. Men were warned to quit their houses when a rat fell from the roof, jumped about as if it was drunk and died. It is not, however, essential to know whether the plague originated in the Central Asia or Central Africa as postulated by different workers. The moot point is that whenever mankind gave up wild living and started to settle as organised families and tribes the zoonotic disease (Plague is only one of them) started affecting them in groups at times following contact with infected animals either in the wild state or during their domestication.

More reliable accounts of plague are, however, found beginning from 200 AD as it appears from the writings of Rufus, the Physician at Ephesus about AD 100. According to him, plague was prevalent in Libya, Egypt and Syria during and before his time, probably as far back as 300 BC (Wu Lien Teh *et al.*, 1936). The more recent happenings are, however, important for understanding the secular trend, the exent and intensities of the problem in the immediate past and the chronological order of events which have led to the present situation.

The Pandemics

Of the three classical pandemics, the *first one* occurred during the reign of the Emperor Justinian (AD 543). It started from Palusium, a great trading centre in lower Egypt from where it spread through North Africa to the Roman Empire on the one side and to Syria, Palestine and Constantinople, on the other, and thence to other parts of Europe and Asia reaching London in AD 662. It prevailed for 50-60 yr and killed about 100 million people.

The *second pandemic* started in the fourteenth century (AD 1347) from Jaffa in Crimea and spread to China and India on the one side and Asia Minor and North Africa on the other. It was imported to Geneva through the Army and from there it spread to other parts of Europe reaching England by 1349. The disease then ravaged Europe for well-neigh three centuries creating horror of what was known as 'Black Death', taking a toll of 25 million human beings. Evidently pneumonic and septicaemic manifestations with bloody sputum, characteristic cyanosis and skin haemorrhages leading to black or blue spots on arms, thighs and other parts, were quite frequent, although the bubonic form must have been more preponderent as for instance in rural England (Greenwood, 1911). Retrogression started in the 17th century from West to East completely leaving the European stronghold by 1841.

This prolonged pandemic in Europe ushered in new changes in the idea as to the causation of disease and its spread and treatment by revolting against the old system and bringing in the new, what is known as *Renaissance* in historical terms. Quarantine laws were first passed in AD 1374 by Count Barnards of Reggio and by the Venetians in 1403, followed by land

Fig. 1. Geographical distribution of human cases of plague in the world (1948-1952).

cordons and sanitary improvements in towns.

Millions died in Asia as well, during this pandemic, and India, too, experienced several epidemics. Starting from the North-Western part of the country it spread to the Central and Southern parts and declined towards the close of the seventeenth century leaving behind endemic foci in the foot hills of the Himalayas in the district of Garhwal and Kumaon where the disease was locally known as "*Mahamari*" and persisted for a long time.

The *third pandemic* (AD 1894) was traced to the plague which reappeared in South China at Yun-nanfu in 1866 from where it reached Canton and Hongkong by 1894. It then spread far and wide through marine transport and involved almost all countries except the main land of Europe by the year 1900. It reached Calcutta in 1895 and Bombay in 1896 and spread from there to almost all parts of India except Orissa, Assam and Eastern Bengal. It prevailed almost unabated till 1918, with a total of 10.25 million deaths. The peak year was 1907 with 1,315,892 deaths (sp. death rate—5.16/1000). From India it spread to Africa and Australasia (1899), Hawaii, Central and South America (1899), to the United States (San Francisco 1900), and to a limited extent to Europe. This pandemic involved almost the whole world. India of course suffered most (Napier, 1946). The spread of infection was rather rapid in this pandemic compared to the slow extension in the second, most possibly due to improved transport and communication facilities. It entered into the United States through San Francisco in 1900. Between this year and 1941 only 501 cases with 316 deaths were reported from eight states which were affected in the following order: California 1900; Washington 1907; Louisiana 1914; Florida 1930; Texas 1920; Oregon 1934; Utah 1930; and Nevada 1937. The great epidemic of pneumonic plague in Manchuria started in 1910 and by 1919 all plague epidemics began to decline everywhere except in Java and East India. A noteworthy feature of this period of decline was the persistence of plague in some endemic foci, particularly in Asia and India and in some cases in wild rodents as in South Africa, California, Iranian Kurdistan, South America *etc.* A fresh spurt of plague was noted during the closing years of the 5th decade of this century and areas affected during 1948-52 are shown in Fig. 1 prepared by WHO.

CHAPTER II

Epidemiology of Plague

From the available historical account it appears that plague visited all regions and countries of the world at one time or the other, barring a few islands and isolated areas, during the course of the known historical period.

World Distribution of Plague

The areas affected by epidemic and endemic prevalence following the third pandemic (Pollitzer, 1954) starting from the epidemic arising at Canton and Hongkong in 1894 are described below.

A. In the Continent of Asia:

(i) China: Starting from Pak-hoi in South-Eastern China in 1866 the disease involved Canton, Hongkong, large territory of Kwangtung province, Fu-Kien, Lui-Chow peninsula, Amoy, Foochow, Chekiang, Kiang-su, Hu-nan, Wenchow, Chaochow and Tao-Chen in Yun-nan province, Shanghai and island of Hainan.

The disease also extended to Nanchang in South Manchuria and from there to North Manchuria, Jehol, Tungliao and Kirin.

(ii) Mongolia and Transbaikalia: Ordos of inner Mongolia (now Sui-Yan) Chahar, Shan-si, Chikti, Shan-tung, An-wei and Kiang-Su provinces, Ningsia and Shan-si.

(iii) Burma: Nam Khan, Mungmao, Rangoon, Meiktila, Pyawbwe, Shan States, extending to South Burma.

(iv) Indo-China: Saigon/Cho-lon to Phnom-Penh of Cambodia (now called Kampuchea), Phan-thiet/Phan-Rang in the South Annam coast and Lang-Bian Plateau.

(v) Thailand: Bangkok, Korat, Northern province of Tak.

(vi) Java: Port of Surabaya, Jogjakarta Surakartaf, middle Java and part of western Java.

(vii) India: Separately narrated in detail.

(viii) Ceylon (now Sri Lanka): Colombo (Port) and other parts of the island.

(ix) Western Asia: (Iran, Iraq, Turkey in Asia and Syria): Iranian Kurdi-stan, Turkis Kurdistan and parts of Mesopotomia, South Iranian Kurdistan,

Bagdad (Iraq) and Syrian border areas.

(x) Western Arabia: Border region of Yemen and Saudi Arabia, mountainous areas of Assyr (forming an outline part of enzootic area in Western Asia).

(xi) Palestine (present Israel): Haifa, Jaffa, Tel-Aviv.

B. Union of Soviet Socialist Republics

Plague in rodents and also in human beings was reported from Siberia, South-East Russia (among *Tarabagan* and *Susliks*), Russian Turkistan, Russian Kurdistan (among Deriones), Trans-Caspian region.

C. Europe

The disease receded from Europe long ago but incidents continued to occur in the Mediterranean regions opposite the African continent. The areas affected were: (i) Corsica and Tarcanto; (ii) Malta; and (iii) Portugal-Azores and Sao Miguel Islands.

D. Africa

North and North-West Africa

(i) Egypt: Ports, upper and lower Egypt *i.e.*, Ports of Said and Alexandria, Ismailia and Suez canal zones, Nile Valley, Coast of Aswan and Province of Asyut.

(ii) Tunisia: Kairouan and Sfax in South Tunisia, Ferryville, Tunis, Bizerte.

(iii) Algeria: Philippeville (Port), Algiers, Oran.

(iv) Morocco: Ports of Casablanca and Rabat, Doukkalle, Agadir and Marrakech.

(v) French West Africa: Dakar, Cape Verde Islands, Western Senegal, St. Louis.

Central Africa

(i) Uganda (Ancient African Centre): Lake Victoria and rural areas.

(ii) Kenya: Mombasa Port, Nairobi, Rongai and rural areas.

(iii) Tanganayika (formerly German East Africa and now Tanzania): Uhehe of Southern Iringa, Kisiba between Kagere River and Lake Victoria, Gasseni district, Eastern slope of Kilimanjaro, Dar es Salaam.

(iv) Belgian Congo: Lake Albert and Lake Edward foci.

South Africa

(i) Union of South Africa: Cape Town, Port Elizabeth, East London, Durban, Pietermaritzburg, King William Town, Queenstown, Johannesburgh, Kitenhage near Port Elizabeth, Orange Free State and Transvaal.

(ii) Basutoland: Enzootic area.

(iii) Bechuana Land: Ngamuland and other areas, Kalahari.

(iv) South West Africa: Gobabis District.
(v) Northern Rhodesia: Luangwa Valley and Barotse Province.

Madagascar
(i) Port of Tamatave, Tananarive Town, Emyrna Province.

E. Australia
The port areas of Sydney and neighbouring towns and ports were involved in the earlier part of the third pandemic, Brisbane (Queensland) and other areas like Rockhampton Townsville *etc.*

F. North America

Western Zone
(i) San Francisco, Los Angeles and Oakland (California); (ii) Seattle (Washington); (iii) Oregon; (iv) Utah; (v) Nevada; (vi) New Mexico; (vii) Arizona; and (viii) Idaho.

South Zone
(i) New Orleans (Lousiana); (ii) Galveston and Beaumont in Texas; and (iii) Pensacola in Florida.

While in the above zones both wild rodent and human plague has been detected, wild rodent plague was also detected in the following states: Montana, Idaho, Wyoming, Colorado, North Dakota, Oklahoma, Kansas and Contra Costa County of California.

Hawaii (USA)
In Oahu Island—Honolulu and other places, Kauai, Maui. In Hawaii Island—Hilo sector, North Hilo, Hamakua district.

G. Canada
Wild rodent plague detected in Alberta and Saskatchewan.

H. South America
(i) Trinidad Islands—North and South American Coast.
(ii) Venezuela—Port La Guaira, Caracas, Miranda and Aragua states.
(iii) Brazil—Santos, Sao Paulo, Rio de Janeiro, Fortaleza, Pernambuco, Santa Maria in Rio Grande de sul, Minas Geraes state. Infection was also detected in wild rodents.
(iv) Argentina: (a) North—Asuncion (Paraguay), Rosario, Buenos Aires, Jujuy, Salta, Santiago del Estero, Tucumen. (b) Central—Rio Negro La Pampa, Mendoza and San Juan. (c) South—Patagonia.
(v) Bolivia—Tarija Department, Vallegrande and Tomina provinces, Santa Cruz and El Palmar (Chaw Boliviano).
(vi) Peru—Ports of Callao, Paita and Mollendo; Provinces of Tumbes,

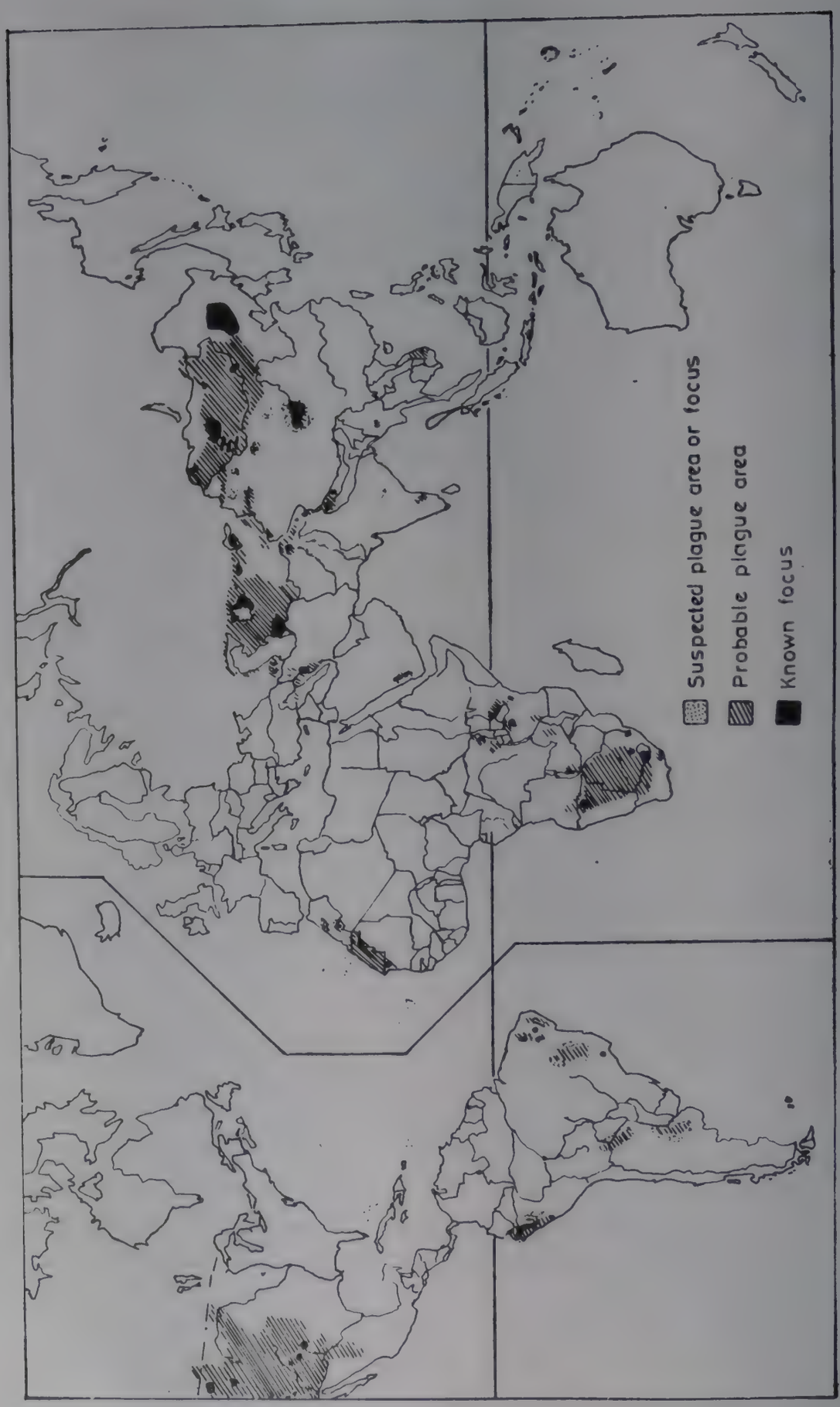

Fig. 2. Geographical distribution of 'human cases of plague in the world reported between 1952 and 1969.

Callao and Moquegua and Coastal Department of Lambayeque), Libertad and Lima, Cajamarca, Chiclayo district.

(vii) Ecuador: (a) Coastal zone: Guayaquil, Province of Los Rioa, El Oro, Guayes and Manabi province. (b) Central zone—Provinces of Chimborazo, Tungarahua and Leon, Canar. (c) Southern zone—Loja Province.

In many of the centres where plague wes present at one time or the other in wild rodents and human beings, the incidence has either ceased or markedly decreased during the 5th and 6th decades (Fig. 2). With the recent advancement of knowledge described in this book, it will be extremely disappointing if the disease is allowed to continue anywhere in the world.

History of Plague in India

The history of plague in India can be conveniently divided into two distinct phases. *viz.* (i) Before the discovery of the plague bacillus in 1894; and (ii) After the discovery of the plague bacillus.

(i) *Before 1894*

1500–600 BC—As recorded in *Bhagvata Purana.*

1031–1032 AD—Plague reached India from Central Asia following invasion of Sultan Mahmoud (Arabian Chronicle).

1325 AD—Plague in Malabar following invasion of Mahmoud, Tuglaq and again after Timur.

1403 AD—Sultan Ahmed's Army was supposed to have been destroyed by plague epidemic in Malwa.

1617 AD—Plague was reported during the Moghul Emperor Jahangir's reign from Punjab, Ahmedabad, Surat, Deccan and a few other parts of India as described by Edward Ferry, Ambassador to the Moghul Court.

1707 AD—Plague occurred in Berhampur—district Ganjam, Andhra Pradesh (now in Orissa).

1812–21 AD—Plague reported from Kathiawar, Gujarat and Kutch—supposed to have been imported from Persia (now Iran).

1836–1838 AD—Plague occurred in Marwar, Rajputana—usually known as 'PALI' plague.

1840–1894 AD—Sporadic cases reported from the endemic foci in the North near Rawalpindi (Punjab) and Kumaon Hills in the district of Garhwal (UP).

1894 AD—Plague bacillus discovered.

(ii) *After 1894*

1895 AD—In Calcutta—diagnosed bacteriologically on the 17th April, 1898 by Dr Neild Cook, imported from Hong Kong.
In Bombay—first diagnosed on the 10th October 1897. From here plague spread rapidly to most parts of India.

1907 AD—Peak year of plague in India with 1,315,892 deaths.

1926–27 AD—Severe epidemic in Hyderabad Deccan.
1947–48 AD—A temporary rise of incidence in several old foci in India.
1948–52 AD—Reappearance of plague in Calcutta (West Bengal), Gaya (Bihar) and in certain places of Vindhya Pradesh (now in Madhya Pradesh).
1954 AD—Plague reappeared in certain places of Andhra Pradesh.
1956 AD—Plague in Guwahati (Assam) first time in the history.
1958 AD—After a small interval plague reappeared in Madras (Tamil Nadu) and Mysore (Karnataka).
1959–61 AD—Plague continued in Tamil Nadu and Karnataka.
1970 AD—A focus of rat plague was discovered in Murarai, district Murshidabad (West Bengal), bordering Bihar.

India got involved very early in the third pandemic of plague in 1895-96, the peak having been reached in about 9-10 years in 1907. Since then the mortality from plague has been one of continuous fall (Table 1 and Fig. 3) except for a small fluctuatory rise in 1960, 1963 and 1965 in the decade

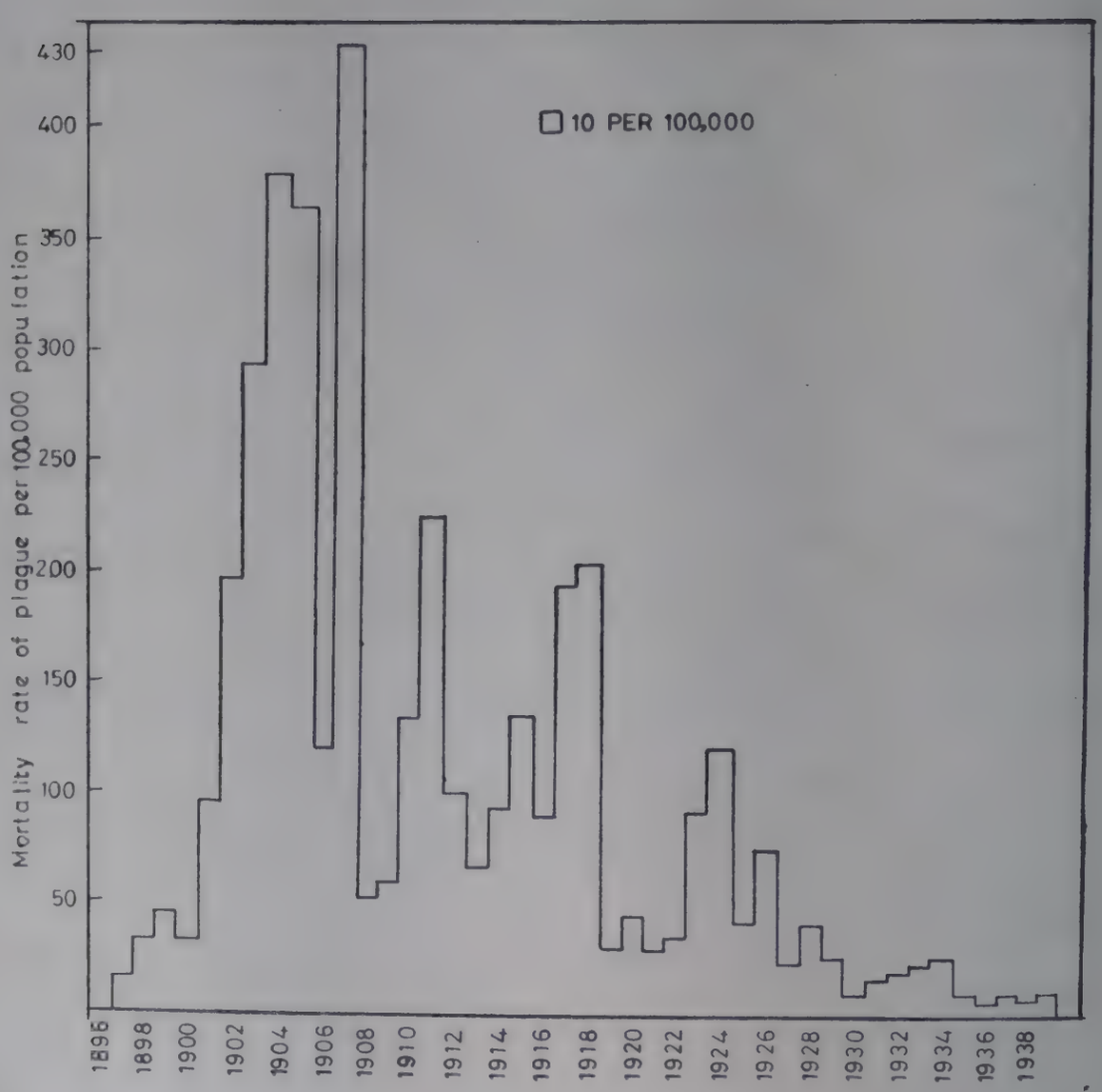

Fig. 3. Plague mortality rate per 100,000 population in India (1896-1939).

Table 1. Mortality from plague in India during the period 1898-1968 arranged in decennially

Period	Total deaths from plague	Total population in each period*	Specific mortality rate/10,000	Plague as % of total deaths 1898-1968	Average annual % of total deaths
1898-1908**	6,032,693	32,91,91,600	183.3	47.47	4.32
1909-1918	4,221,529	31,55,92,638	133.8	33.22	2.32
1919-1928	1,762,718	32,83,19,580	51.9	13.40	1.34
1929-1938	422,880	36,19,45,872	11.7	3.33	0.33
1939-1948	268,596	39,65,92,490	6.8	2.11	0.21
1949-1958	590,059	32,87,64,907	1.8	0.46	0.55
1959-1968	942	46,83,64,958	0.2	0.001	0.01
	1,32,99,417	—	—	—	—

*Population was first calculated for each year by inter-census correction and then added together for different periods. Since 1948, population of the areas forming Pakistan was excluded.

**The first period taken was of 11 yr.

1959-1968 (Table 2). Actually no death from plague has been recorded in India since 1969.

Table 1 shows that starting from a specific mortality rate of 183.3 per 100,000 population during the first decade of plague pandemic in India, the mortality came down to 0.2 per 100,000 in the decade ending in 1968 and to nil in the last decade 1969-1978, and India has remained free from plague thereafter. The average annual deaths due to the disease as percentage of total annual deaths came down from 4.32 to 0.01 by 1968 and nil after 1969. It must however be mentioned that although there was a continuous decline of incidence and deaths due to plague, since 1939 these fluctuated quite alarmingly between 1943 and 1948 and as high as 78,937 human deaths due to this disease occurred in India in 1947 as compared to 10,577 deaths in 1942 (Table 2). Among the states, plague virtually disappeared from Bihar, Maharashtra, Madhya Pradesh (including the old Vindhya Pradesh) and West Bengal following 1953, but it persisted in Mysore till 1968 and in Tamil Nadu till 1964 and there was revival of the disease in Andhra Pradesh between 1954 and 1963 and cases were also reported from Madhya Pradesh, Himachal Pradesh and Rajasthan in 1966 and 1967 before final disappearance.

Plague gradually receded from India after 1969. The question that still remains unanswered is weather it is due to certain measures taken or it is an interlude in the secular trend in the epidemiological behaviour which such diseases have often exhibited in the past. It is on the correct assessment of the situation that the nature of the steps to be taken now or in future will depend.

The distribution of plague deaths on statewise basis between July 1898 and June 1932 is given in Table 3. The statewise records of plague after 1932 was irregular because the states were reorganised several times particularly following independence in 1947. However, the changes that occurred between 1939 and 1968 have already been noted in Table 2.

Table 3 shows that the main brunt of onslaught prior to 1933 fell upon three states namely, Punjab, Bombay and UP followed by Bihar and Orissa, CP, Hyderabad and Mysore (of British period) in serial order. The mortality per 1000 mean population was highest in the first three states mentioned above. Moreover, the fatality rate prior to the discovery of sulpha drugs and streptomycin was extremely high. For instance, in the first Calcutta epidemic, according to Crake (1908), the fatality rate varied between 91.2 and 94.5 per cent among the Hindus and between 94.2 and 96.2 per cent among the Muslims and 51.3 to 53.3 per cent among the Christians. In the absence of specific remedies much however depended on the standard of living, nourishment, facilities for medical care and nursing and perhaps also on racial characteristics. For instance, in the Hong Kong epidemic the case fatality among the overcrowded, indifferently fed and unclean Chinese amounted to 93.6 per cent while it was 77 per cent among the Indians, 60 per cent among the Japanese and only 18.3 per cent among the Europeans. The results were similar in Calcutta. The chance of recovery was better in men

Table 2. Annual deaths from plague in different States in India during 1939-1968

Year	Andhra Pradesh*	West Bengal	Bihar	Maha-rastra	Madhya Pradesh	Madras	Mysore	UP	Punjab and Haryana	Rajasthan and Others	Total	Rate/ 100,00
1	2	3	4	5	6	7	8	9	10	11	12	13
1939	6758	0	1938	1472	852	324	2352	21662	0	0	26257	6.88
1940	1500	0	1040	5573	283	1169	2593	11725	0	0	19799	5.13
1941	2713	0	129	5311	761	1725	5417	4035	0	22	11984	3.08
1942	657	0	108	680	129	701	3776	8953	0	6	10577	2.67
1943	1408	0	266	715	144	4885	3882	7556	0	11	13578	3.38
1944	5263	0	834	2514	410	1738	5357	15454	61	14	21525	5.29
1945	6631	0	1523	11779	595	1644	8016	14024	61	13	29751	7.21
1946	4026	3	8089	3405	189	2254	2894	18206	203	6	32997	7.84
1947	1791	11	13204	3081	2902	3078	1502	51455	246	8	78937	18.61
1948	811	18	2142	1305	2860	978	1128	13722	1905	16	23191	7.02
1949	2103	57	2155	1139	3495	151	982	9875	211	19	20197	5.78
1950	719	3	1449	140	5568	42	255	10231	241	201	18813	5.33
1951	98	8	3	1	475**	60	542	12595	3	12	14178	3.97
1952	1	16	3	2	457**	12	272	3107	0	20	3905	1.08
1953	18	0	0	0	541**	6	56	762	0	0	1385	0.378
1954	28	0	0	0	0**	0	115	157	0	0	296	0.08
1955	14	0	0	0	0	1	137	29	0	0	194	0.052
1956	0	0	0	0	0	1	52	5	0	0	74	0.019
1957	0	0	0	0	0	0	15	1	0	0	17	0.0044
1958	0	0	0	0	0	4	9	0	0	0	9	0.0021
1959	1	0	0	0	0	9	7	0	0	0	12	0.0028
1960	0	0	0	0	0	22	16	0	0	0	25	0.0054
1961	2	0	0	0	6	17	31	2	0	0	56	0.013

(*Contd.*)

Table 2 *Contd.*

1	2	3	4	5	6	7	8	9	10	11	12	13
1962	5	0	0	0	0	6	78	0	0	0	100	0.022
1963	2	0	0	1	0	1	17	0	0	0	25	0.0054
1964	0	0	0	0	0	0	108	0	0	0	109	0.023
1965	0	0	0	0	0	0	386	0	0	0	386	0.079
1966	0	0	2	0	1	0	46	0	0	30	87	0.017
1967	0	0	0	0	2	0	71	0	0	22	95	0.018
1968	0	0	0	Case only 0	0	0	46	0	0	0	46	0.0088
1969	0	ϕ	0	0	0	0	0	0	0	0	0	0.0
1970	0	0	0	ϕ	0	0	0	0	0	0	0	0.0

*Figures for the years 1939 to 1952 relate to Hyderabad only.

ϕIndicates no death.

**The corresponding figures for Vindhya Pradesh (included in MP by amalgamation) were 186, 14, 18 and 20 respectively for these years. Assam had only 2 deaths in 1956, the other years recording only 5 cases with no death. Himachal Pradesh recorded 8 deaths in 1966; from 1967 to 1970 only 5 cases with no deaths were recorded; prior to 1966, no records are available.

These records are based on the Annual Report of DGHS, India and WHO Statistical Reports. The discrepancies noted in the total may be due to reshuffling of the states at different times.

Table 3. Province-wise distribution of plague mortality between July 1898 and June 1932

Province	Mean population census	Total plague deaths	Per cent of All-India total	Mortality/ 1000 of mean population
Punjab	21,142,793	3,489,123	28.7	165.0
Bombay	19,877,756	2,460,132	20.2	123.8
United Provinces	47,164,594	2,911,837	23.9	61.7
Bihar and Orissa	34,692,676	1,113,937	9.2	32.1
Central Provinces	13,991,863	468,165	3.8	33.5
Hyderabad	12,855,934	425,302	3.5	33.0
Mysore	5,970,446	314,673	2.6	52.7
Rajputana	10,330,957	282,312	2.3	27.8
Madras	42,168,483	227,184	1.9	5.4
Central India Agency	7,653,893	149,941	1.2	19.6
Burma	5,970,446	149,427	1.2	11.8
Other areas	38,477,465	109,597	0.9	2.8
Bengal	46,109,157	68,809	0.6	1.5

than in women and the disease assumed less severity in the vaccinated than in the unvaccinated population.

Plague Epidemics in India

Regional Foci and Periodicity

The historical review shows that plague, allowed to pursue its natural course, assumed a pandemic form at times and spread in different directions originally from the endemic homes of Central Asia or Central Africa and involved distant parts of the globe. It then receded to the original home leaving behind islands of endemic centres in the various parts of the world, particularly in sub-mountainous areas and depending upon the means of communication, nature of reservoirs and vactors and conditions favourable for the reception it took a century or more to reach distant parts of the globe. A number of centuries passed before the disease showed signs of regression and it also took more or less an additional century for the process to be completed. With faster and more frequent means of communication this behaviour also changed and the spread of infection in the last pandemic became accelerated manifold. The important point is that the areas last involved were usually the first to recover unless they presented favourable grounds for sylvatic or natural plague. The endemic areas of plague in India depicting the foci of natural plague were defined by Sharif (1951). He suggested

that the infection was entrenched in three groups of foci in northern, central and southern India (Fig. 4) namely:

(a) the northern foci consisted of three endemic centres at the foot of the Himalayas, perhaps forming part of a big sub-Himalayan focus. These centres were considered responsible for plague outbreaks in East Punjab, Uttar Pradesh (formerly United Provinces) and districts of Bihar north of the River Ganga.

(b) The focus in Central India (Madhya Pradesh) comprised the water sheds of the Vindhya, Bhanrer and Maikal ranges and the Mahadeo hills.

(c) The three southern foci were situated in: The water sheds of Western

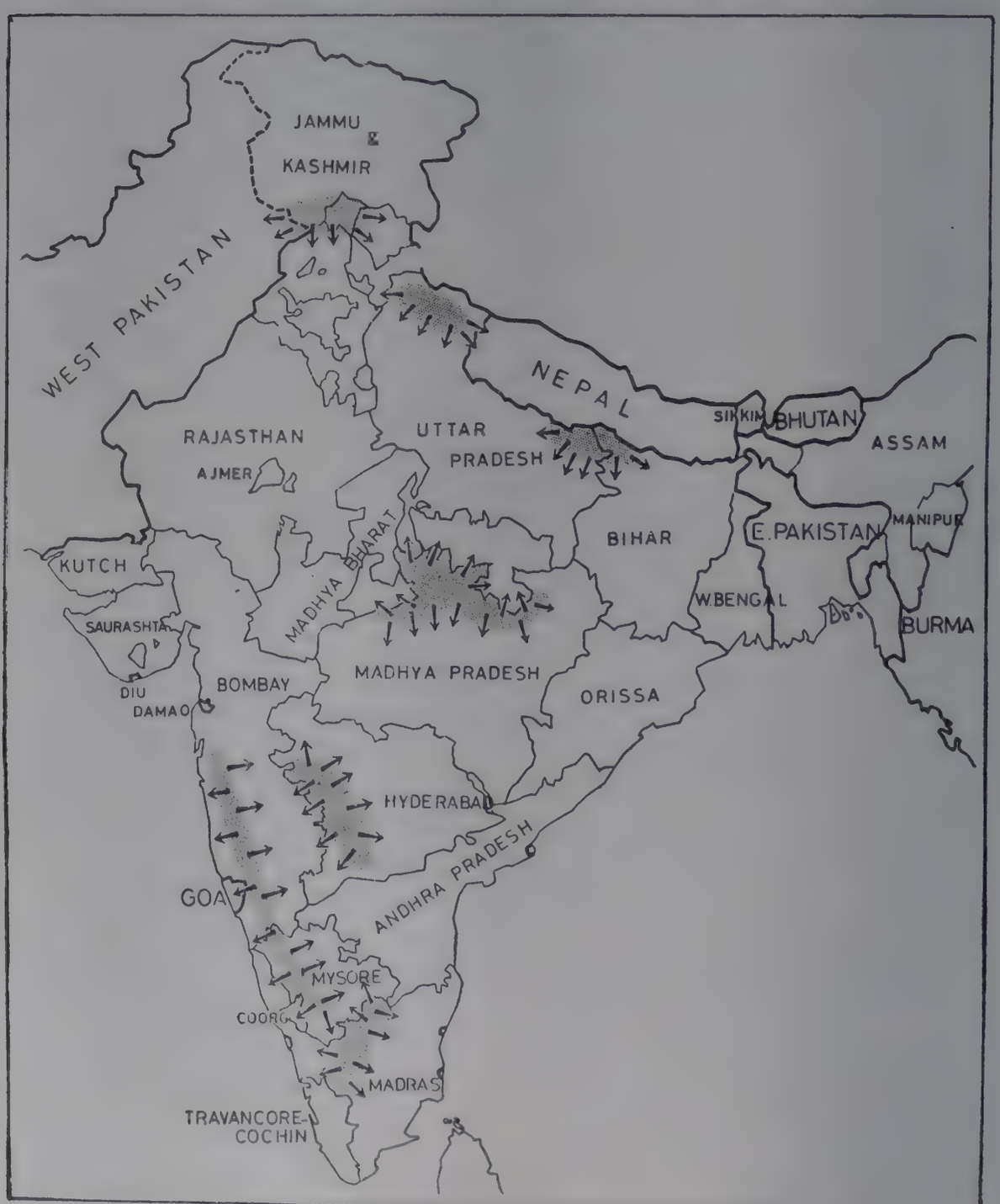

Fig. 4. Endemic plague foci in India according to Sharif (1951). Arrows indicate the direction of radiation of plague and not actual course of the progress.

Ghats of Bombay and Mysore States; water sheds located in the districts of Salem, Coimbatore, Nilgiri and Madurai in Tamil Nadu; and the hilly regions of Andhra Pradesh, particularly Matanchari and Cumbum Valley.

The endemic centres in Southern India may have been established after the city of Bombay became infected in 1896. Maharashtra and Karnataka states and the adjacent parts of Andhra Pradesh were involved in 1898, the Nilgiris in 1903 and Hyderabad city in 1911, when the plague situation became rather serious. On the other hand, it seems possible that the endemic foci in the Himalayas were of long standing, plague infection being known in the Kumaon and Garhwal districts since 1823. The infection might have persisted there as a relic of the great pestilence of the seventeenth century and might have been responsible for occasional plague outbreaks until 1877. Thereafter it remained latent for sometime, to become active again early in the present century. In Central India and Madhya Pradesh plague started after the infection in Bombay in 1896. The endemic foci described by Sharif are supposed to be the result of this importation of the infection, but it is difficult to say whether the existence of all these foci can be substantiated by factual data.

After 1939 the main epidemic feature noted is the gradual reduction in the number of plague deaths in the earlier part of the period, followed by an increase in its peak reaching in 1943 in Tamil Nadu, in 1945 in Maharashtra, Andhra Pradesh and Karnataka, in 1947 in Bihar, Uttar Pradesh and Punjab, in 1949 in West Bengal (around Calcutta only) and in 1950 in Madhya Pradesh. No plague death has been recorded in Punjab and Bihar since 1952, in West Bengal, Maharashtra and Andhra Pradesh since 1953 and in Vindhya Pradesh (part of Madhya Pradesh now) since 1954, but it persisted in Tamil Nadu, Karnataka and Andhra Pradesh for a few years more with new invasion in Assam and revival in Himachal Pradesh and Rajasthan lasting for one to two years only. It appears to have finally ceased from 1969 without any death from plague being reported till now (1984).

During pandemic prevalence the disease exhibited epidemicity of varying degrees in different areas depending upon the local conditions. Occasionally in the inter-epidemic period plague was introduced into a non-endemic area but failed to establish itself and fizzled out in a year or two as in Assam in 1957 (Seal and Bose, 1957). Thus plague exhibited two types of secular periodicity, *viz.*, (i) long-term periodicity with intervals of centuries and continuing for centuries or part of a century when once started at such intervals, *e.g.*, recurrence of pandemic plague in the sixth, fourteenth and twentieth centuries; and (ii) short-term periodicity—a tendency to renew at short intervals. This is apart from concurrent seasonal periodicity.

The phenomenon cannot be explained in terms of fortuitous happenings or ephemeral local conditions. It rather comes as an outcome of the working of the biological laws. It would be wrong to regard squalor, poverty and absence of sanitary safeguards as the determining cause of pandemic

rise because neither are these confined only to plague epochs nor are they peculiar to plague infected countries. It is insufficient to ascribe it to spread of infection along a trade route that has probably been operative since earliest civilisation, while it cannot be denied that the direction and rapidity of spread may be effected by the prevailing methods of transport and communication. The belief expressed by some workers that ousting of the black rat by the brown was the cause of decline of the second pandemic can only be partially substantiated although the history of plague in Bombay, Calcutta and other parts of India shows that these two species can live together in amity and play their part with fine impartiality in determining the occurrence of plague epidemic of great intensity. What we have found is that whenever the proportion of black rat as also of *X. cheopis* was reduced or lessened due to better housing and sanitary conditions, the incidence of plague had also come down or became greatly reduced, giving a clue to its control and eradication. A comparative study of ward 8 (plague affected) and ward 10 (plague unaffected) in Calcutta by the author during 1954–60 also supported this view.

Again, that the emergence of pandemic is due to a gradual rise of the infection quantum to the epidemic flash point has so far remained as a speculation based on probabilities rather than on experimental proof. Similarly it is a surmise that the decline of pandemic and its gradual retreat to its indigenous home or old (primary) focus is dependent upon the rise of herd immunity as a result of which the disease disappeared first from those areas where the conditions were least favourable to its persistence and from other parts of the world outside its indigenous home. According to this assumption the essential cause of the long-term periodicity or of pandemic plague is ascribed to the rise and fall of herd immunity of rodents rather than to the recurrent happenings and emphemeral local circumstances. It may be mentioned here that in 1948–49 plague not only reappeared in Calcutta alone but also in many other towns and cities where plague was absent or at least quiescent for a long time *e.g.*, Bombay, Lucknow, Dhanbad, Asansol *etc.* Simultaneously a tendency to recrudescence in places like Hyderabad, Mysore, Rajasthan, Madhya Pradesh and Himachal Pradesh had been noted. The problem is how these events or phenomena can be explained or interconnected.

In the author's experience a probable explanation is that when an infection reaches almost to a stage of extinction the organism undergoes a mutation in virulence or toxicity or both as a biological process for the preservation of species. By this procedure the effect of rise of immunity or resistance against the existing strain in the community is overcome so that the freshly emerging organism may then have another base of life through the epidemic or pandemic which it causes. But the question is how the inter-epidemic period is actually briged over in plague. This was, however, the subject of study by the author during the period 1954–57 which will be elaborated in Chapter VI.

Recent Epidemics of Plague in India

The epidemic waves of plague occurring in Calcutta and Howrah (West Bangal), Gaya (Bihar), Guwahati (Assam), Madras (Tamil Nadu) and Mysore (Karnataka) between 1948 and 1962 have been studied (Seal, 1949; Seal and Prasad, 1959; Seal and Patnaik, 1963). Their general features on the basis of knowledge gained during the first half of the present century (Phase IV) are described below:

(*i*) *General Characteristics of the Epidemic Waves*

A typical epidemic curve is generally symmetrical differing with normal curve in regard to the height of the peak. In the pre-epidemic period it takes some time to develop epizootic among the field and sewer rats (*R. norvegicus*) which may be of sylvatic origin. It takes about 10 days for the epizootic to appear in the house rats and about another 10 days to develop in human beings. This relationship is well seen in Fig. 5.

In warm climate the wave is generally symmetrical, of long duration and low pitch. In northern India (substropical) the crescendo is less abrupt than diminendo, amplitude is great and duration relatively short. The extent of human plague is solely dependent upon the intensity of infection and mortality among house rats (except in case of pneumonic plague epidemic). The post-epidemie period usually follows that among rats, during this period the interaction of several factors, particularly deaths of the susceptibles, rise of immunity in the remaining rat population and climatic factors playing upon them and the fleas, bring about a temporary decline (inter-epidemic period).

The peculiarity of plague epidemics particularly in cities and towns is that after its introduction or reappearance, it goes on for a few years before a time interval is reached (Fig. 6), and usually bigger the city the longer is

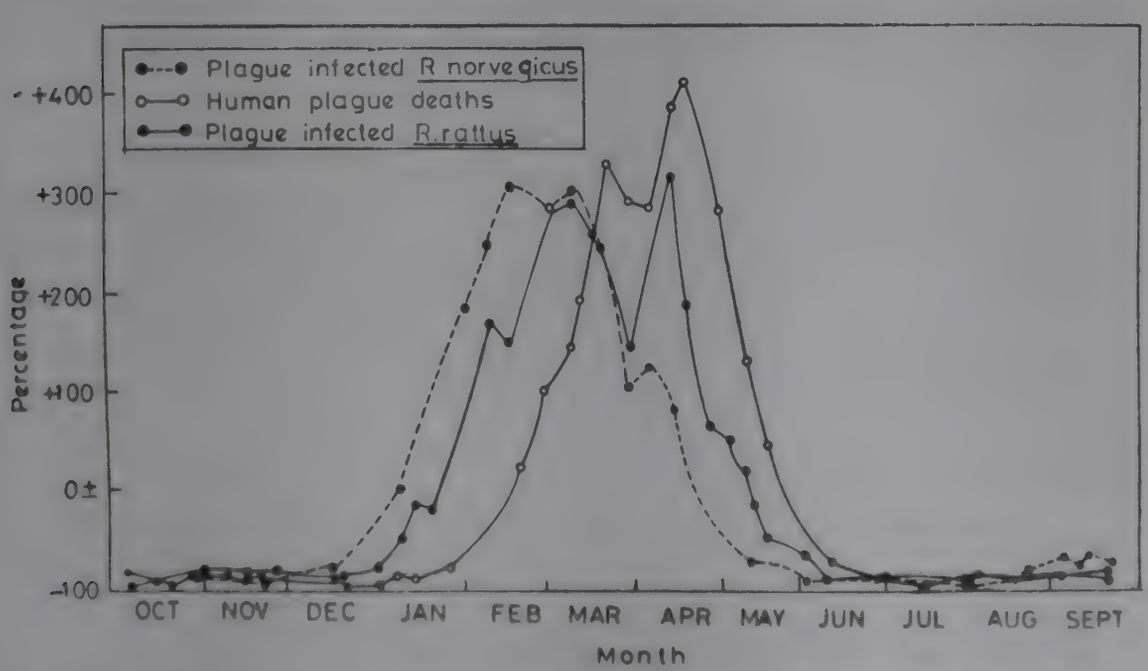

Fig. 5. Sequence of epidemic plague in rats and men (British Plague Commission).

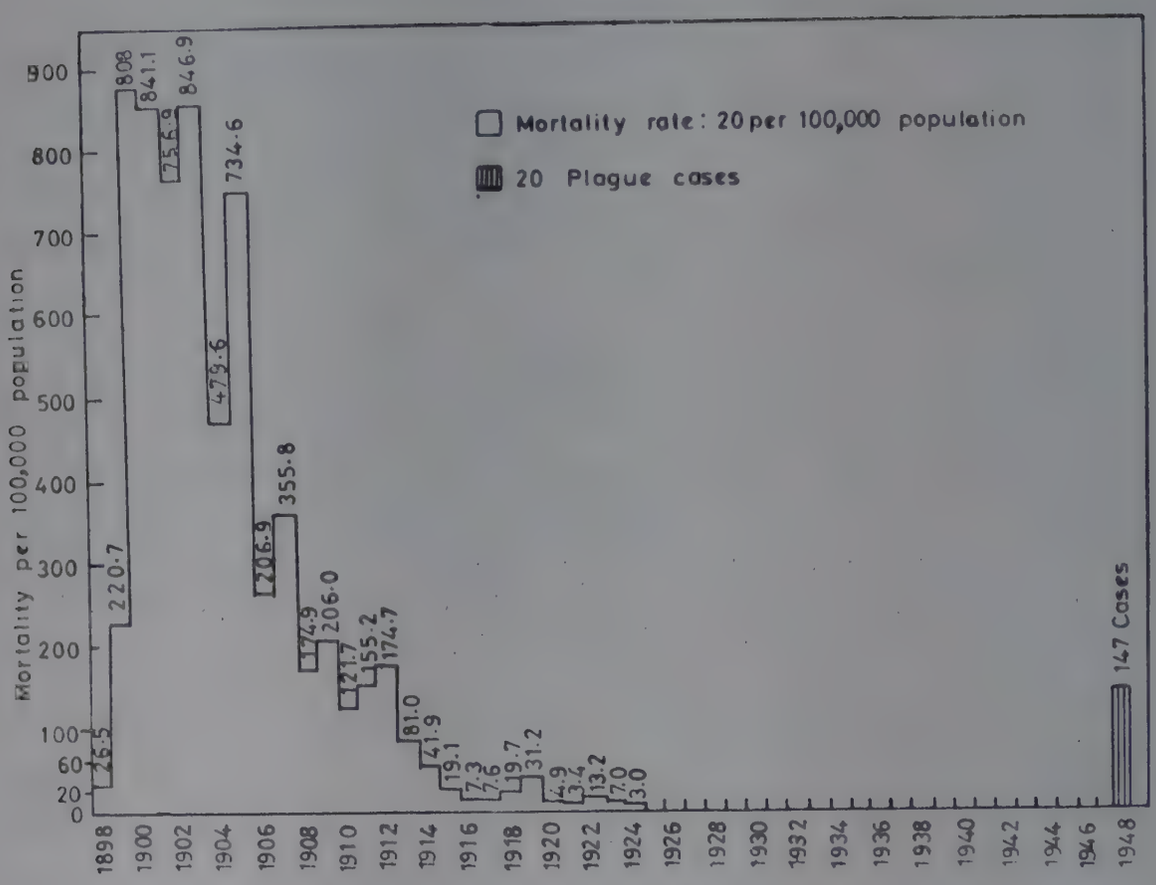

Fig. 6. Plague mortality in Calcutta (1948-1952).

the range of continuity. It is also noticed that in the beginning small outbreaks occur which gain momentum within 3 or 4 yr unless seriously interfered within its earlier phase of first 2 yr, and then a heavy epidemic occurs for some years followed by gradual fall through several years till its disappearance for some time. This character of the epidemic is probably due to what is considered as 'incomplete' epidemic. The infection quantum gradually rises during the winter and early spring along with high flea and rat infestation and low herd immunity; the sudden onset of hot weather near the end of April causes a rapid fall in atmospheric humidity in northern India with a premature cessation of transmission. The abrupt decline of epidemic wave is considered as "incomplete" epidemic because it terminates before the herd immunity has reached the climax (Fig. 7).

In a big city like Calcutta where there is enormous rat population it takes several years for a large size epizootic to develop, which is maintained for several years due to almost continuous supply of susceptibles in this tropical climate, and again it takes several years to reduce the susceptibles in sufficient number (thus raising the herd immunity) and to bring about a cessation. Concomitantly, following a big human epidemic the herd immunity among human population also rises. In this phenomenon, the role of the parasite concerned (*Y. pestis*) may be equally important. This is what has been studied in the Calcutta epidemic to be described in the following pages under clinico-epidemiological studies in plague during 1948-1952 in Calcutta.

Morbidity: Cases are generally severe in the early phase of the epidemic.

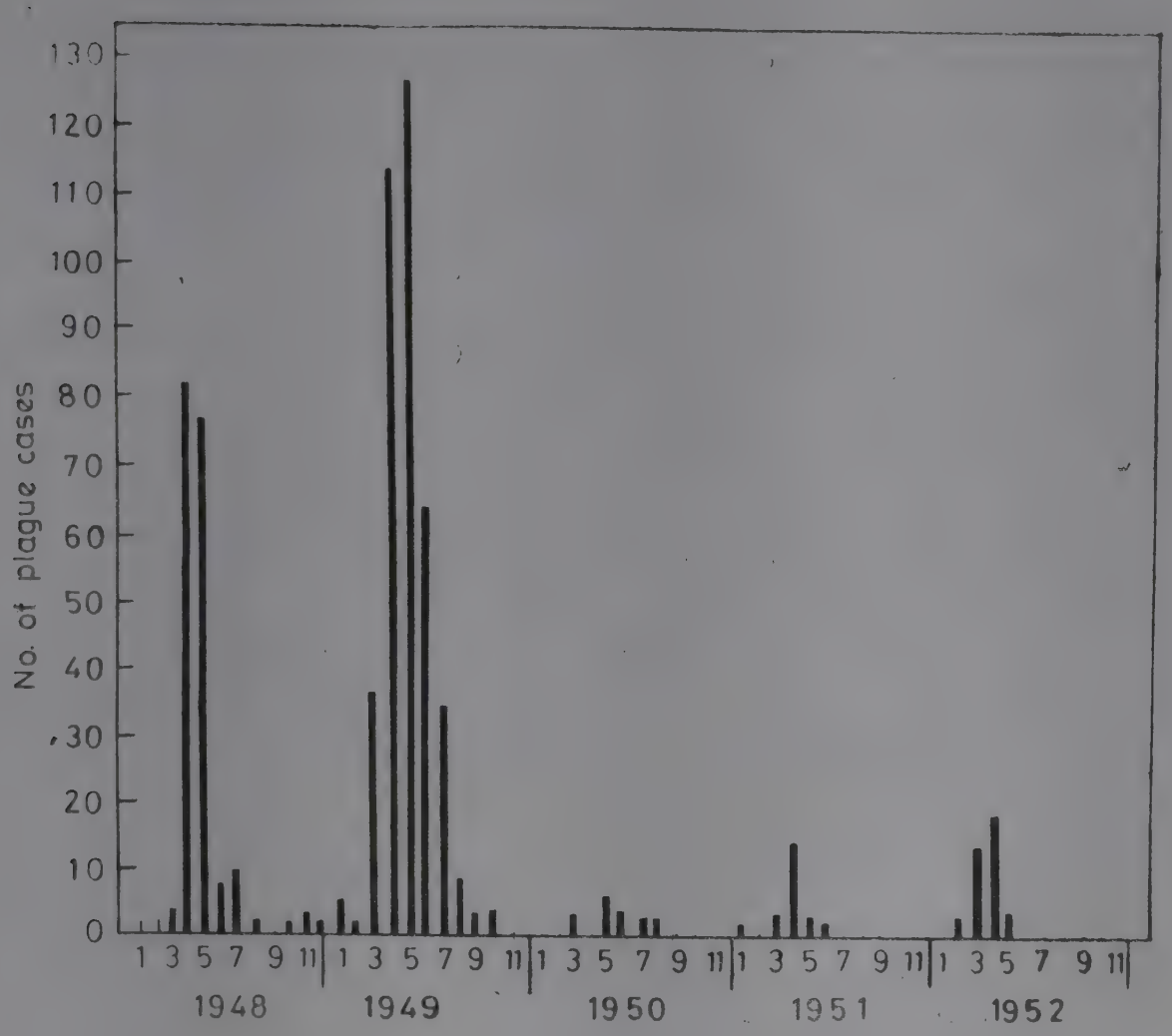

Fig. 7. Plague cases in Calcutta (1948-1952).

With the progress of the epidemic, severe cases become less and finally are of mild type showing only slight fever and bubo. People of all age groups except infants (0-6 months old) may get the disease. Sex and occupation generally have little influence but in Calcutta epidemic three-fourth of the cases were males. One of the reasons may be the high proportion of males compared to females in the city population, the M : F ratio being 63.7 : 36.3 at the time of the epidemic.

Mortality: The case fatality rate in bubonic plague varies in different epidemics. It is usually greatest at the beginning and height of the epidemic, the fatality rate varying between 60 to 90 per cent in untreated cases. Much depends on the clinical condition of the patients and medical and nursing care available. While in the Hongkong epidemic the case fatality rate among the overcrowded, indifferently fed and unclean Chinese amounted to 93 per cent, among the Japanese it was 60 per cent, among the Indians 77 per cent and among the Europeans only 18.3 per cent.

Spread: It usually spreads by contiguity but it may also spread to distant places through infected rats or fleas being carried with commodities or in persons body or clothes and also from one country to another through maritime or aerial transport. In a city the first appearance of plague in a locality may be followed by a rise in small foci (as in grain stores, warehouses *etc.*)

before covering wider areas. It has predilection to dirty, insanitary dwellings more particularly in the ground floor attacking generally poorer class of people in the rat infested huts and bustees. Granaries, store rooms, broken store dumpings and disposed refuse and dustbins attract and harbour rats and thus form foci for the spread of infections.

(ii) *Urban-Rural Distribution*

Plague is both urban and rural in India, the latter predominating. It appears that plague failed to gain a foothold in many of the towns in India, perhaps owing to the unfavourable climatic conditions and the lack of any efficient flea vector (as in Assam, Madras town and Bengal particularly in the areas inundated annually by floods as in East Bengal (now Bangladesh) largely destroying the field (peri-domestic) rats. Assam did not have it due to absence of vector fleas. Another factor which may play an important role is the distribution and proportion of various types of rodents. Given a suitable flea vector, a large proportion of *R. rattus* will make for easier and quicker spread of plague among human beings than a similar proportion of other rodents. On the other hand, replacement of one rodent by another as in Bombay, may disturb the balance and plague may recrudesce or be imported with consequent severe outbreaks. The development of natural immunity among commensal rats is another factor which may prevent an outbreak or make for simmering enzootic plague. However, given a suitable climate, a sufficiently large rodent population, effective vectors and the plague bacillus the infection may often become firmly entrenched among the rats of towns or cities and persist there for many years as in Bombay and Calcutta. If such towns or cities are of commercial importance, having traffic connections with the towns and rural areas, they might spread plague infection and create secondary foci or plague distributing centres for further propagation of the disease.

The epidemiology of rural plague differs from that of urban plague in one essential respect namely, that the rural plague may be dependent upon infection of commensal, peri-domestic or even wild rodents and is usually initiated by importation of the infection. The persistence of plague in rural areas remained as a problem of study in India. Kunhardt (1912) differentiated the role of 'incomplete' and 'complete' plague. Kunhardt and Chitre (1921) also worked out a scheme for predicting the carry over of epidemics, but Strickland (1933) while agreeing with the principle pointed out with much reason (Pollitzer, 1954) that the figures arrived at were not generally valid. However, the recent observation made by the present author in connection with the urban plague suggest that there is every possibility of infection being carried over by the rat population during the inter-epidemic periods, possibly independently of the size of the locality or of the rat community. And, indeed, there are villages in the Cumbum Valley where plague used to appear year after year (George and Webster, 1934). In fact, it is the same phenomenon as we observe in human and other animal population.

Thus the rural plague may not only be the result of importation from plague affected urban centre but the contrary may also be equally possible *i.e.*, plague map spread from an affected rural centre to the towns through grain traffic or by means of other vehicles and communications as observed by Pollitzer (1954). Again plague may shift from one village to a neighbouring one and can come back to the first village after 2 or 3 years interval. Plague workers have put forward the notion of area-wise endemicity to explain the phenomenon. According to Sharif (1951) the slow type of epidemic, killing fewer rats persists longer than the severe epidemic causing heavy rat mortality. On the basis of observation in Uttar Pradesh Busvine, (1957) suggested that plague was not endemic in U.P. but it shifted from one place to another through infection of contiguous colonies of field rodents, eventually infecting the commensal rats of a village on its path, thus causing a rat epizootic and human plague. In fact, multiple factors are involved and the same factor may not be equally operative in all places. At least three essential factors must occur together for epizootic plague, which may or may not be followed by human cases: (i) presence of the plague bacillus in the active or passive reservoirs; (ii) susceptible rodents; and (iii) an efficient vector species. Climate and season play a secondary role.

Role of wild rodents: In Manchuria, Mongolia and South-East Russia no intermediary was needed but fleas fed on *tarabagan* could successfully transmit the infection to guineapigs and domestic rats. In Dakar (Senegal) multimammate mouse and in South-East Russia *M. wagneri* played intermediary role. In Ecuador people reared up guineapigs for meat and often got infected. Rats in California sometimes got infected from the ground squirrel. In Bombay (India) Sharif found infection among wild rodents like *Tatera indica.*

Sylvatic plague: From various considerations it would appear that the main centres of sylvatic plague were in the Central Asian Plateau although Mongolia, South-Eastern Russia and Tibet had endemic centres. Similarly in Central Africa there were ancient centres with their own areas of influence but not as old as the Asian centres. The Californian sylvatic area differed from others in that the infection was originally contracted from *R. rattus.* The same is probably true of the South African *gerbils* which derived the infection from rat plague originating in ports. In Argentina and Ecuador (South America) also the wild guineapigs were probably similarly affected.

Sources and mode of infection: In India, *R. rattus*, the house rat, was the commonest source of human infection. These rats in their turn, received it from the sewer rats, *R. norvegicus* or *Gunomys varius* (*B. bengalensis*). During the inter-epidemic period the infection persisted in a simmering state as chronic infection among them. When the circumstances like climate, herd susceptibility or biological position became favourable, the quantum of infection rose among them and was soon transferred to the house rats and evantually to human beings. The role of these rats in the endemic or the infected area was found to be roughly as follows: *R. norvegicus* dead 22 per

cent, live 0.85 per cent; *R. rattus* dead 16.7 per cent; and live 0.01 per cent.

Spread of plague among rats: Rats suffer from both acute and chronic plague. Greater the number of acute infected (septicaemic) rats the greater is the number of human cases. Carrier immune rats are found in the endemic areas (60% Bombay rats were found immune whereas 90% of Madras rats were susceptible). Again the various species of *R. rattus* differ in their habits and ectoparasites; mice is less susceptible to plague than rats as their usual flea is not *X. cheopis* but *Leptopsylla musculi* which shows little tendency to attack men. But in some places and circumstances they may play a part in plague epidemic.

Intramural spread: *R. norvegicus* may often play a bigger role in the spread of infection than *R. rattus* but the spread may be very slow and irregular. Infection is often spread through carrying of infected fleas or rats. Alternate flooding and draining of rice fields generally cause rat migration and spread of plague. The point of importance therefore is the proportional distribution of the types of rats and their densities.

Relation of rats to chronic plague: The presence of bacteraemia is needed for flea infection and it was suggested that the infected flea maintained the intramural infection rather than the rats suffering from chronic plague. This point therefore needed investigation and is reported later (Chapter VI).

Rat fall and some characteristics behaviour of dying rats: In an epizootic, the infection among rats often spreads rapidly and they begin to die at odd hours of the day and night, *R. rattus* actually falling from the roof, rat runs or other nesting places. They are often seen to come out into the open or to the floor of a house, make erratic movements and may even scream before dying. When such a behaviour is seen in a rat particularly with swollen neck and inguinal glands, plague should be at once suspected.

Seasonal incidence of plague in rats: The seasonal peak of the epizootic among rats occurs generally in the following order: in Bombay and Calcutta —February-March; Lahore—March-April; Jhelum—April-May; Rawalpindi—May-June; further north—July-August-September. The immunity and herd resistance in the rat population also change accordingly.

It may thus be maintained that the climatic factors, by reason of their influence upon the transmission of infection are capable of determining the season of the year in which plague epidemics would most likely occur. The season influences the numerical prevalence and longevity of rat fleas and multiplication of the plague organism in both rats and fleas (vide experimental observations made by the author in Chapter VI). The temperature in rat burrows is generally 3-10°F lower than that of outside atmosphere, and rats rarely develop septicaemia below 50°F (10°C).

At high latitude the atmospheric temperature attains the critical level only during the late summer and early autumn so that it is at this season of the year that a plague epidemic is liable to occur at that latitude. A decrease in latitude was therefore associated with earlier seasonal occurrence. In the sub-tropical region, on the other hand, where either the temperature

or the humidity factor is unfavourable during summer, plague epidemic had a vernal periodicity (as in Northern India).

An analysis of the first 20 years' records of plague epidemics in different parts of India since 1896 suggests two principal seasonal manifestations of epidemic intensity in a year. First, there was a broad-based single wave, starting in autumn or late autumn and rising to a peak in March or April. In this category were generally included the provinces of Northern India with Punjab in the North-West and Bengal in the North-East. Bombay city also fell in this category. Secondly, there were double waves which could be further sub-divided as follows: (a) a main autumnal wave with a secondary rise in the early months of the year, *e.g.*, Hyderabad and Mysore State; (b) a main spring or late spring wave with a secondary rise in the autum *e.g.*, Kashmir and North-West Frontier Province; (c) almost equal wave in the autumn and spring *e.g.*, Bombay city and State (Maharashtra), Central Provinces and Central India (Madhya Pradesh); and (d) a main peak occurring in the early month of the year with an occasional secondary rise in the autumn, *e.g.*, in Madras (Tamil Nadu) state (only few cases in the city of Madras; Fig. 8).

In brief, the peak of the spring wave was delayed more and more as one moved from the Bombay area towards the north, north-west or any highest altitude (*e.g.*, Kashmir), and also there was a tendency for the double wave to give way to the single one. On the other hand, the peak comes earlier as one moved towards the South or South-East. For instance, in some outbreaks in North-West Frontier Province the peaks occurred in May or even in June, whereas they occurred in January or February in Madras (Tamil Nadu). The two months which showed the least incidence in India were June and July and also January and February, where the winter is severe.

In Calcutta, out of 20 epidemic waves since 1896, four reached their peaks in March, twelve in April and four in May. In the whole of Northern India, the common experience was that the maximum plague mortality occurred at the end of the cold season and the disease tended to die out with the onset of the hot weather, because at that time the humidity was inimical to the survival of the rat flea and to its power to transmit plague infection. The most favourable period of the year was therefore autumn and spring, particularly the latter, when the fleas were seen to come out of the rodent burrows, when great epidemics of bubonic plague were liable to occur.

Reappearance of plague in Calcutta: Plague infection was first brought to Calcutta from Hongkong through maritime route and it ravaged the city for well-neigh 25 yr with peak in 1907. It occurred sporadically until 1925 when the last case was recorded and reappeared in March 1948 in the same Colootola area close to the dock where it originally appeared in 1895. But the first few cases were probably missed and the case that was referred to the hospital on the 17th April was bacteriologically diagnosed as a case of plague on the 19th April, 1948. Even so, it took considerable time to convince the local authorities to take immediate measures, resulting in rise

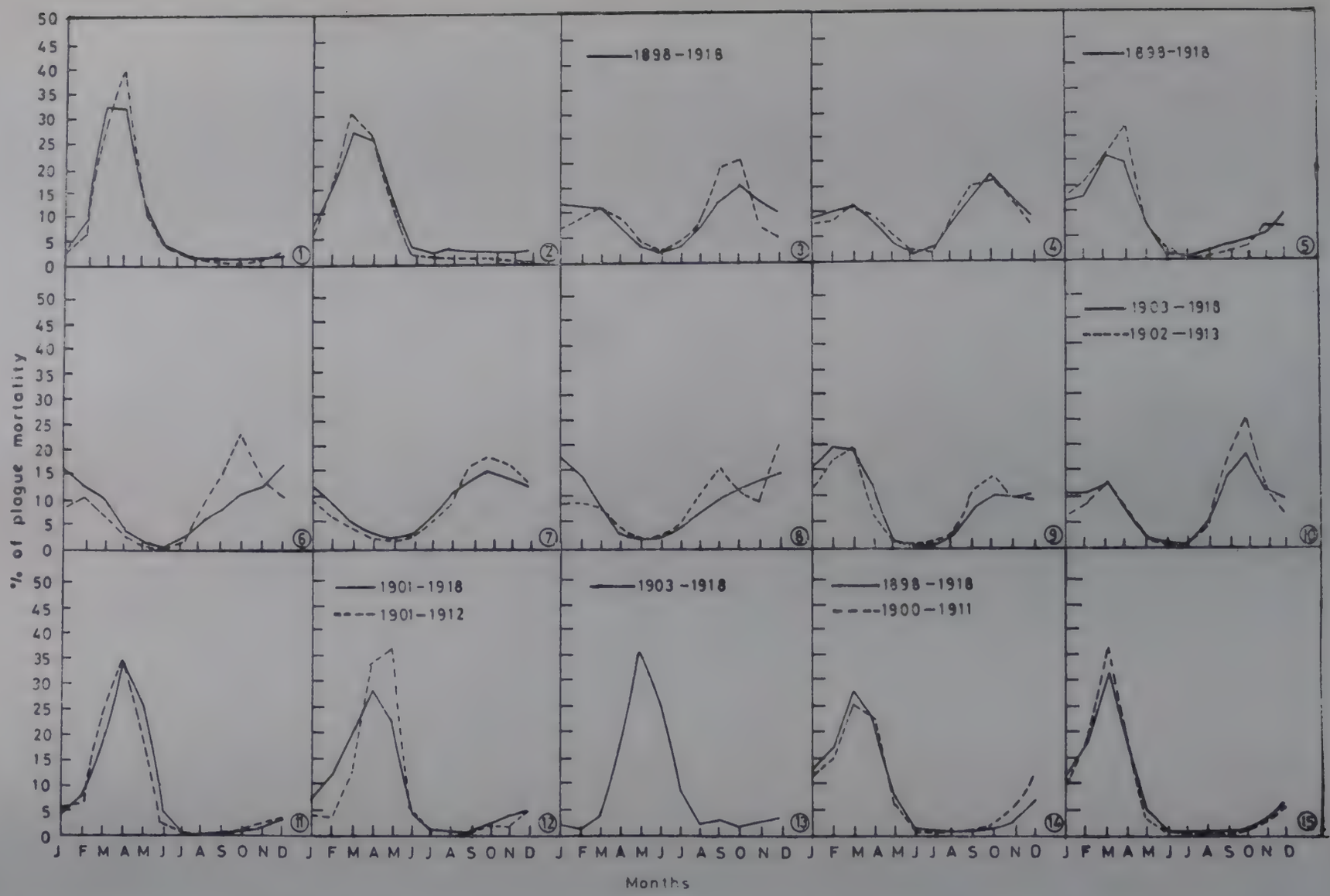

Fig. 8. Seasonal distribution of plague mortality in different cities and provinces in India—— = mean for 1899-1918; - - - - = median for 1900-1911. 1. Calcutta city; 2. Bombay city; 3. Bombay Province; 4. Bombay Native State; 5. Rajputana states; 6. Hyderabad State; 7. Mysore State; 8. Madras Province; 9. Central Province; 10. Central India; 11. Punjab Province; 12. Jammu and Kashmir State; 13. N.W.F. Province; 14. United Province; 15. Bengal.

of the epidemic with greater vigour next season—February, 1949 with peak in May of that year. No case was recorded after May 1952 (Fig. 7) perhaps due to the active measures taken under continued surveillance.

Incidence of plague cases: The total admissions including 15 cases otherwise detected were 812 during the entire period (1948-52). The majority of cases (687) was from Calcutta (84.6%), 50 (7.4%) from Howrah (across the river Ganges) and 65 cases (8.0%) from the neighbouring areas. Definite clinical evidence was present in 650 (80%) cases which included 15 pneumonic and 207 (44.1%) bacteriologically confirmed cases. Altogether 93 persons died giving a case-fatality rate of 14.3 per cent.

Distribution of Bubonic Plague in Different Parts of India

Guwahati (Assam)

Except for some cases imported during the exodus from Calcutta in the earlier part of the first plague epidemic in 1902-04 no human or rat plague was ever reported in Assam. In 1948 plague was suspected due to heavy deaths among the rat population in Darrang in North Lakhimpur district of Assam regions, but on investigation by the author (Seal, Ghosal and Bose, 1951) it proved to be deaths due to heavy and prolonged rainfall flooding the rat burrows. In November, 1956, however, epizootic plague was detected by the author (Seal and Bose, 1957) in the town of Guwahati, the gateway of the State. Following this enzootic plague, 4 human cases with two deaths were recorded.

Here, there was the virgin terrai for the development of the infection, but prompt and extensive application of DDT and prophylactic immunization prevented any epidemic in the following year or thereafter, but at least 5 cases were reported from another area on way to Shillong from Guwahati. The only possible clue as to how the infection might have been introduced was that a large consignment of mustard seed in gunny bags had arrived in Guwahati by goods train from a town in Uttar Pradesh. The first human case, which had remained unreported, was one of the labourers sleeping inside the godown where the seed bags were stacked, and the first known case occurred in the house of the godown owner, The infected flea carried through the merchandise was suspected but no flea could be collected although four of the dead rats out of seven and two of the live rats out of 13 yielded *Y. pestis*. There were only four human cases, three in one house and one in a neighbour's house, all bubonic and yielded evidence of *Y. pestis* infection either by culture of bubonic material or blood at the Pasteur Laboratory, Shillong. Intensive anti-plague measures undertaken quickly brought the epizootic to a stop and no further human case was reported.

South India (Tamil Nadu and Karnataka)

Although human cases of plague ceased to be reported from practically all parts of India by the year 1960 slow grade of endemic prevalence still

continued in the state of Tamil Nadu and Karnataka. This led to an on-the spot investigation by the author and his colleague (Seal and Patnaik, 1963) in 1960.

Tamil Nadu: At the time of investigation in 1961, 9 districts of Tamil Nadu out of 12 were involved in endemic prevalence of plague (Fig. 9). The reported attacks and death from plague in 9 districts of the state between 1940 and 1960 are given in Table 4.

Table 4 shows that the most affected districts were Salem, Nilgiri, Coimbatore, North Arcot and Madurai in serial order of these again, plague ceased to appear in human beings in North Arcot and Madurai since 1950, in Coimbatore since 1951 and in Nilgiris since 1953. It also shows that

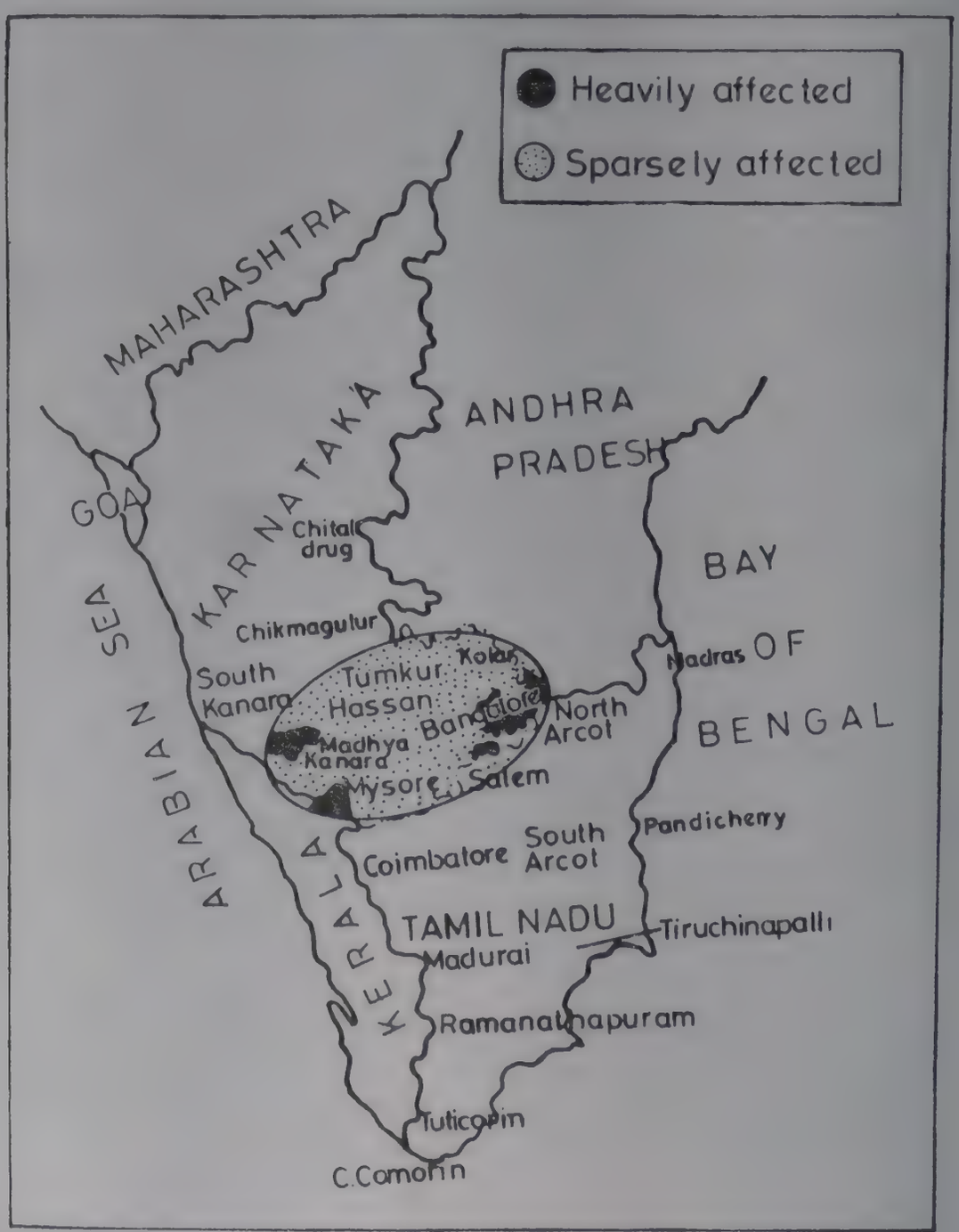

Fig. 9. Plague endemic areas in Tamil Nadu and Karnataka States (old Madras and Mysore) (1959-1961).

within the period of 20 yr (1940-1960) the peak years were 1943 and 1948, the maximum number of cases being 7464 with 5100 death in 1943. Salem was the only district which persistently remained endemic, particularly involving its Hosur Taluka.

Figure 10 shows the seasonal distribution of plague in Hosur Taluka depicting four periods *viz.*, 1926-36, 1936-45, 1945-55 and 1956-60. It reveals that although cases were occurring more or less throughout the year two district peaks were discernible, one in February-March and another in September-October.

With regard to anti-plague measures, official records showed that the control work was not undertaken with the thoroughness necessary to prevent

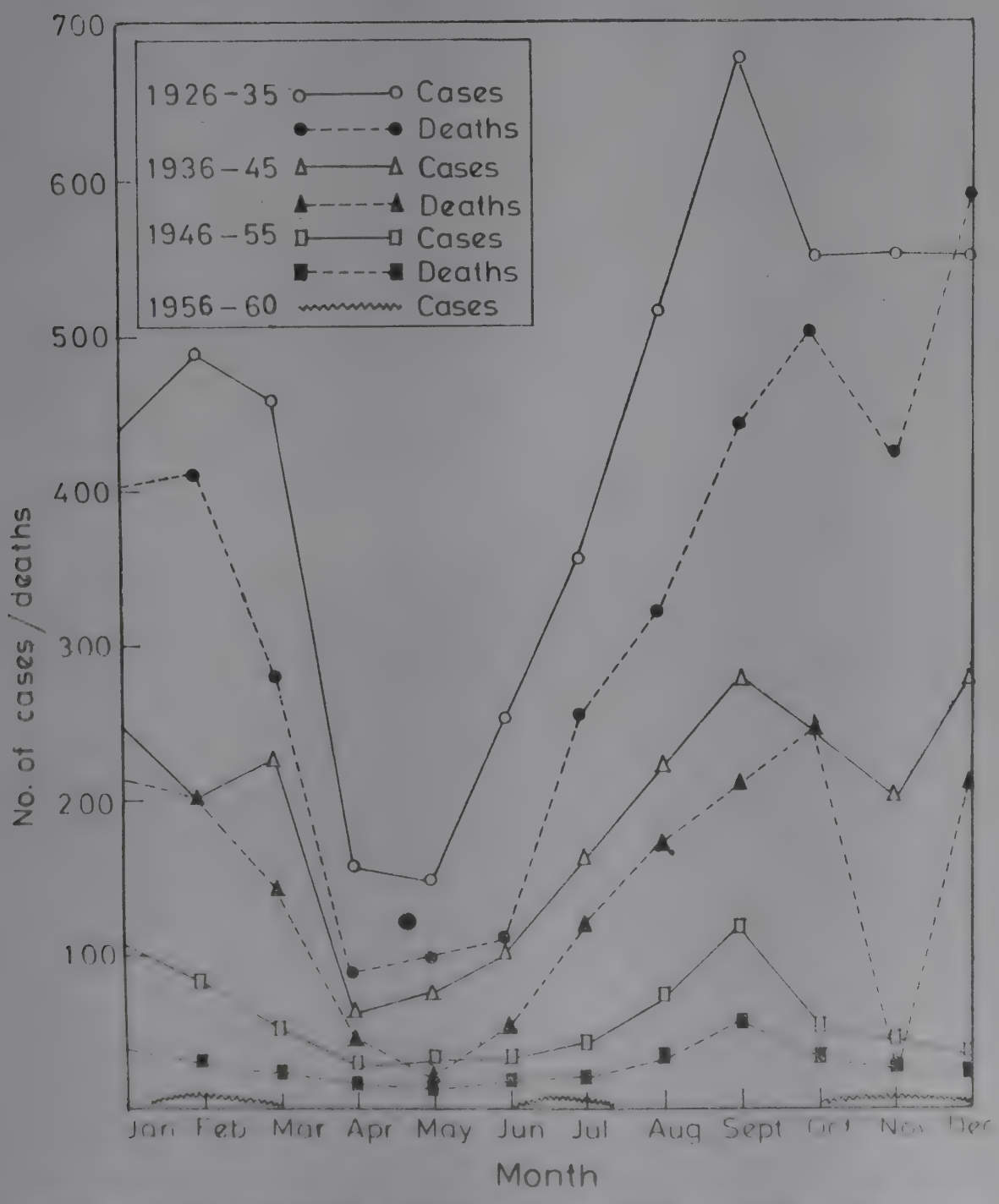

Fig. 10. Seasonal incidence of plague in Hosur Taluka of Madras (Tamil Nadu) State (1926-1960).

Table 4. Reported attacks and deaths from plague in

Year	Salam		Nilgiri		Coimbatore		North Arcot		South Arcot	
	A	D	A	D	A	D	A	D	A	D
1940	386	184	15	11	219	76	—	—	—	—
1941	295	106	88	11	148	50	13	11	—	—
1942	254	125	111	51	160	74	4	3	—	—
1943	463	239	92	45	6876	4796	18	15	—	—
1944	341	163	61	34	1673	1184	32	22	—	—
1945	529	303	370	163	1294	1076	40	35	58	22
1946	297	187	153	60	3577	1996	57	30	57	22
1947	376	179	117	18	2901	1495	676	454	280	120
1948	285	132	66	13	401	294	40	19	67	25
1949	166	68	29	8	39	7	28	18	—	—
1950	3	1	7	4	2	—	—	—	—	—
1951	6	5	—	—	—	—	—	—	3	2
1952	18	10	5	2	—	—	—	—	—	—
1953	9	6	—	—	—	—	—	—	—	—
1954	8	6	—	—	—	—	—	—	—	—
1955	—	—	—	—	—	—	—	—	—	—
1956	2	1	—	—	—	—	—	—	—	—
1957	4	1	—	—	—	—	—	—	—	—
1958	1	—	—	—	—	—	—	—	—	—
1959	17	7	—	—	—	—	—	—	—	—
1960	29	14	—	—	—	—	—	—	—	—

A—Attacks; D—Deaths; The districts of Ramanathapuram, Tirunelveli

Table 5. Attacks and deaths from human plague cases in the

Year	Bangalore		Chitaldurg		Chikkamangalore		Hassan		Kolar	
	A	D	A	D	A	D	A	D	A	D
1950	27	11	91	29	2	—	35	17	24	13
1951	42	9	26	7	4	2	43	20	49	17
1952	41	14	—	—	—	—	58	22	79	36
1953	—	—	—	—	—	—	9	6	39	19
1954	4	—	—	—	—	—	2	1	294	107
1955	25	7	—	—	—	—	—	—	417	115
1956	28	9	1	1	—	—	—	—	130	36
1957	5	2	—	—	—	—	—	—	29	11
1958	—	—	—	—	—	—	—	—	5	1
1959	—	—	—	—	—	—	—	—	8	2
1960	—	—	—	—	—	—	—	—	29	7

districts of Madras State between 1940 and 1960

Madurai		Tanjore		Trichurapali		Chingleput		Total	
A	D	A	D	A	D	A	D	A	D
6	4	—	—	—	—	—	—	626	275
—	—	—	—	—	—	—	—	544	178
3	1	—	1	—	—	—	—	532	255
15	14	—	—	74	—	—	—	538	5109
20	18	—	—	—	—	2	1	2129	1422
17	10	—	—	—	—	—	—	2308	1609
15	7	—	—	—	—	—	—	4156	2302
579	266	12	8	22	9	1	1	4964	2510
217	93	17	9	—	—	—	—	1093	585
6	2	15	8	—	—	—	—	283	111
—	—	—	—	—	—	—	—	12	5
—	—	—	—	7	6	—	—	16	13
—	—	—	—	—	—	—	—	23	12
—	—	—	—	—	—	—	—	9	6
—	—	—	—	—	—	—	—	8	6
—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	2	1
—	—	—	—	—	—	—	—	4	1
—	—	—	—	—	—	—	—	1	—
—	—	—	—	—	—	—	—	17	7
—	—	—	—	—	—	—	—	29	14

and Kanya Kumari had no attacks and deaths due to plague.

districts of Mysore State between 1950 and 1960

Mandya		Mysore		Shimoga		Tumkur		Total	
A	D	A	D	A	D	A	D	A	D
20	4	252	148	62	21	40	10	553	253
3	4	628	296	42	5	282	97	1119	457
20	7	404	190	—	—	8	1	610	270
3	—	83	35	1	—	—	—	134	60
—	—	7	2	—	—	—	—	307	110
—	—	—	—	—	—	—	—	442	122
—	—	6	3	—	1	—	—	185	50
—	—	4	1	—	—	—	—	38	14
—	—	4	2	—	—	—	—	9	3
—	—	12	3	—	—	—	—	20	5
—	—	35	3	—	—	—	—	64	10

any future outbreaks. In spite of the 5 teams working the main defects seemed to be in respect of (*i*) absence of an epidemiological unit in thes tate for the purpose of field investigations and guiding the control measures; and (*ii*) systematization of control measures to cover every village with several rounds each year without waiting for the plague to arise *i.e.*, undertake full-fledged surveillance operation.

In Karnataka State: Prior to reorganisation of the state of Mysore (the present Karnataka) in 1952, the State consisted of 10 districts of which 9 districts were affected with plague till 1950. Fortunately the new districts added in 1952 were plague free. By 1960 the Kolar and Mysore districts continued to have plague cases until control measures were reorganised following the investigation by the author and his colleague in 1961.

The attacks and deaths from human plague in the various districts of Mysore state during 1950-1960 are given in Table 5 and deaths between 1938 and 1960 in Fig. 11. It will be seen that most of the districts became practically free from human plague cases by the year 1952, which continued in Hassan till 1954 and in Bangalore till 1957. However, Mysore and Kolar continued to have cases beyond this period. Deaths due to plague in Karnataka State between 1938 and 1949 are shown in Fig. 12 and also between 1938 and 1960 in Fig. 11 indicating the endemic character of the disease.

The seasonal occurrence of plague in Mysore State between 1938-49 is shown in Fig. 12. There were two peaks, one in January and the other in

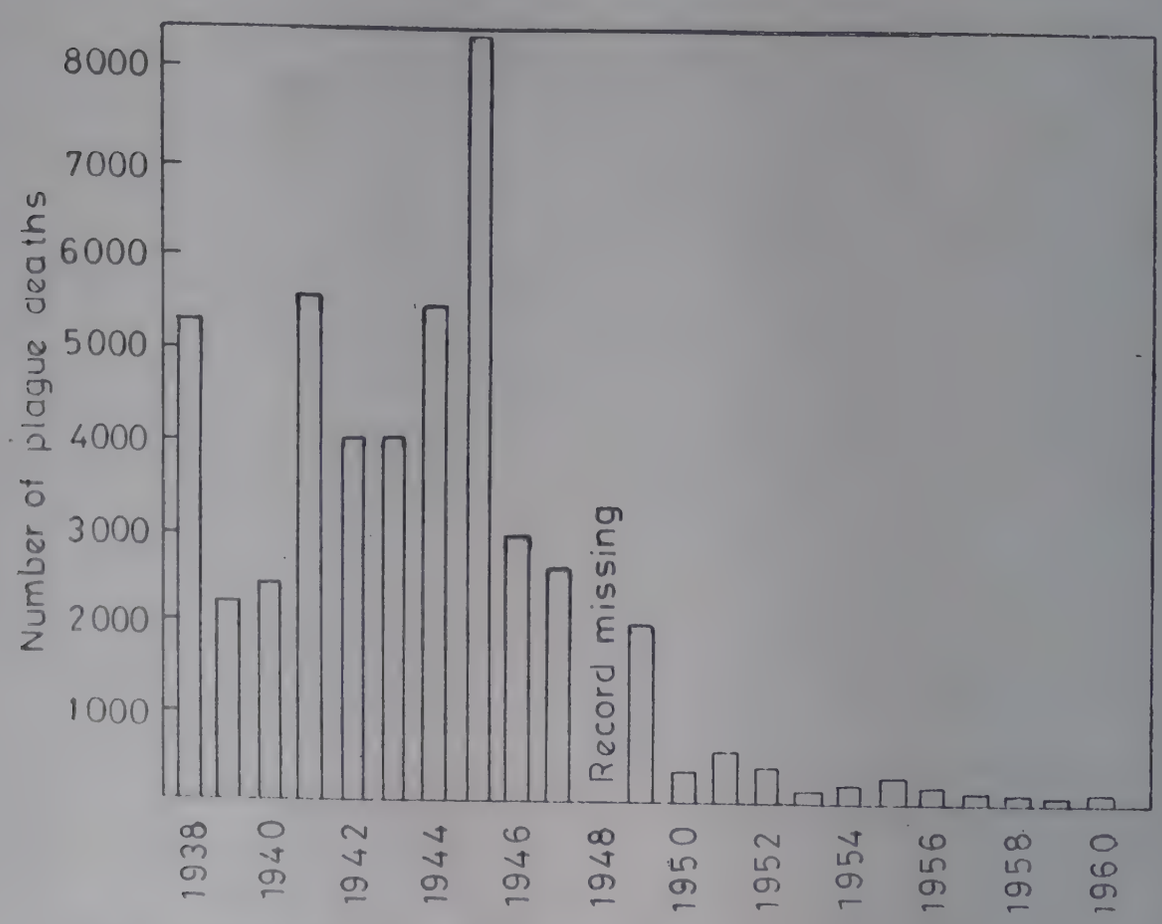

Fig. 11. Deaths due to plague in Mysore (Karnataka) State (1938-1949).

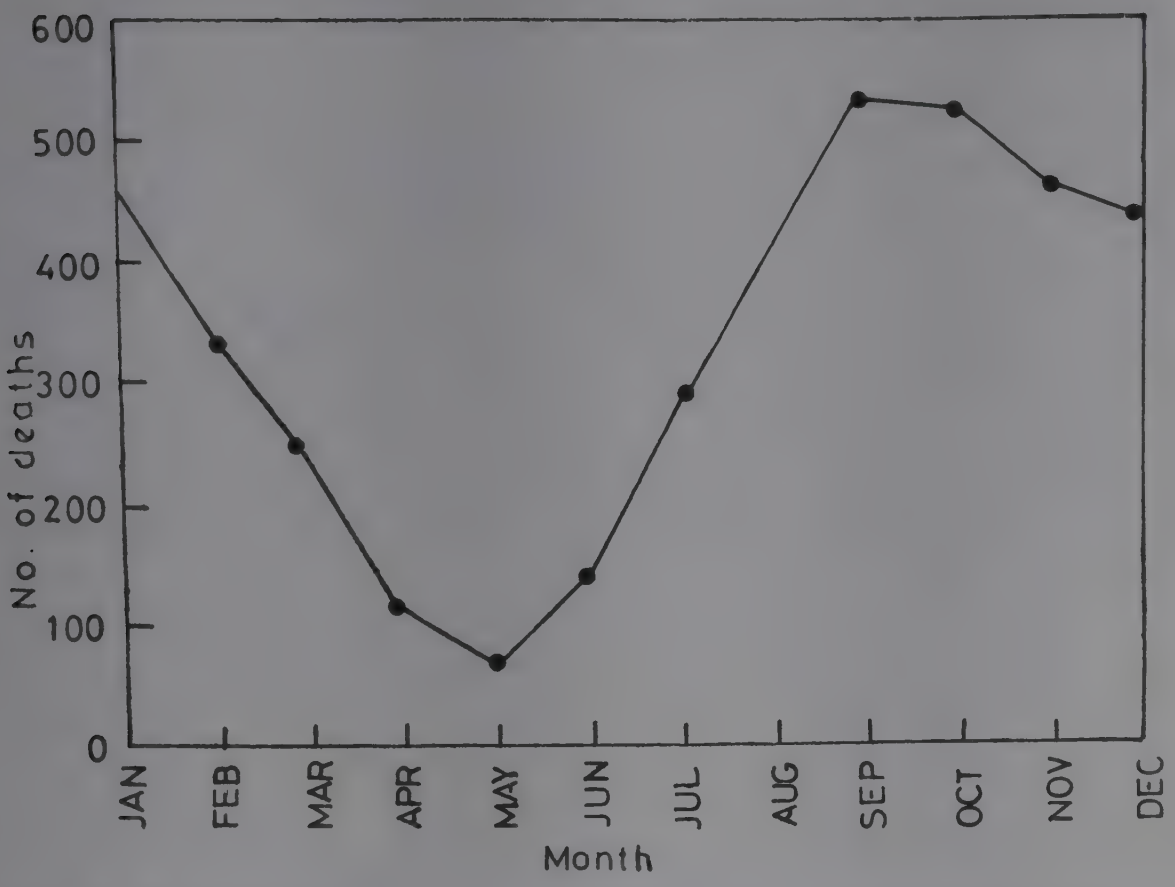

Fig. 12. Deaths from plague per month in Mysore (Karnataka) State (1938-1949).

September. The Karnataka State occupies a position in Table 1 and immediately adjoining Eastern Ghats. The chief water sheds lie in the North-West in and around Nandidurg having a height of 4851 ft above sea level, radiating 7 rivers in all districts. The maximum temperature is 30-33°C in April-June and minimum 16-18°C in December-January. The average rainfall is 29-30 inches (about 72.5 cm). The monsoon period extends from mid-July to mid-November.

As part of anti-plague measures, both rats and human cases were sporadically examined for plague organisms. Rats and fleas were found positive every year between 1956 and 1961. DDT insufflation was also undertaken but in 1958-59 fleas were found to become resistant.

The combined experience of the area involved in the two states (Karnataka and Tamil Nadu) showed (Fig. 9) that although the areas had fallen within the political boundaries of the states, as far as plague is concerned, the two areas should have been considered as one geographical unit and hence the so-called allegation of plague being introduced from one state to another had no validity.

The main weak links of the anti-plague campaign in the two southern states can be summarized as follows:

(*i*) Sufficient importance was not given to the problem of plague and no concerted coordinated efforts had been made to study the problem intensively so as to adopt adequate measures either to eradicate or to prevent plague, by any one of the states.

(*ii*) Only partial attempts were made to suppress the vector fleas leaving

the reservoir rats to flourish unrestricted in the face of possibility of resistance developing among the vector fleas.

(*iii*) The operation of the programme was mostly limited to official agencies alone and not much effort had been made to create sufficient interest and consciousness among the local public for cooperation and assistance.

(*iv*) No epidemiological unit was employed for investigation and planning for adequate control.

The situation was finally tackled by (*a*) formulation of principles of eradication by a thorough investigation of the problem; and (*b*) necessary central assistance offered for total eradication through a joint committee representing both the states and centre. The area has been free from plague since 1968.

Clinico-Epidemiological Studies of Plague in Calcutta (1948-1952)

Plague was introduced into Calcutta first time in 1895 through maritime route from Hong Kong and took heavy toll of lives till 1917. Thereafter only sporadic cases occurred till 1925. After a lapse of about 23 years it reappeared in its original focus in March, 1948 and continued in the city till 1952 with peak incidence in 1949 (Fig. 7). No human case has been reported since then but some evidence of rodent infection was obtained till 1958 and thereafter till 1982 (as reported by the surveillance team still under operation) no rodent infection has been detected. The following containment measures were taken during the period of epidemic and latter during surveillance.

1. Establishment of a laboratory for bacteriological investigation of human cases and rat infection and management of surveillance work.
2. Rat control by catching, trapping and baiting of live rats and collection of dead rats for dissection, bacteriological examination and study of density and distribution of rats.
3. Destruction of rats on a mass scale by cyanogassing and other means.
4. Control of fleas by disinfestation with DDT spraying of houses and rat shelters.
5. Setting up of an emergency isolation camp hospital for accommodation of patients and specific treatment.
6. Immunisation of public by antiplague vaccine.
7. Improvement of garbage disposal to prevent rat harbourage.
8. Clinico-epidemiological investigation and research on various aspects of the disease problem, particularly on the problem of transmisson, spread and inter-epidemic behaviour.

The antiplague control and investigation work had to be extended to the following areas beyond the city limit namely, (*i*) East and South Indian Railways, (*ii*) Port Commissioner's areas, (*iii*) Howrah—the city across the River Hooghly and several adjacent Municipalities like South-Suburban, Garden Reach, Dum Dum South and North, Barrackpur and Kamarhati. Later the control operation had to be extended to Greater Calcutta area

involving Alambazar, Baranagar, Uttarpara, Chandannagar, Bally, Belur, Hooghly, Jagatballavpur, Ichhapur, Chakdah, Dum Dum Airport, Central Jail and Fort William. Actually the Epidemic Diseases Act of 1895 was promulgated, which made the people more conscious of the situation and offer their cooperation in the control work.

Incidence of Plague Cases

The particulars of the total number of confirmed, probable and possible cases detected between 1948 and 1952 are given in Fig. 13.

The total number of probable cases was 812 during the period 1948 to 1952 including 15 cases otherwise detected, 650 or 80 per cent of these cases were clinically diagnosed and 44.1 per cent bacteriologically confirmed. Cases were of three clinical types namely bubonic—612 or 94.2 per cent, septicaemic—22 or 3.4 per cent and pneumonic—16 or 2.4 per cent. The fatality rate was 14.3 per cent including 14 deaths among 16 pneumonic cases (14 cases of familial outbreak in a village 16 miles away from Calcutta and 2 familial cases in Calcutta). Among the pneumonic cases only 2 survived, one in the village and one in the city.

In this familial outbreak of the total number of cases (14), 8 were males and 6 females, 12 familial and 2 extra familial. The patients included a compounder of the local doctor and an old nursing woman from the neighbouring house. All patients were adults (20-60 yr old) except one four year old female child.

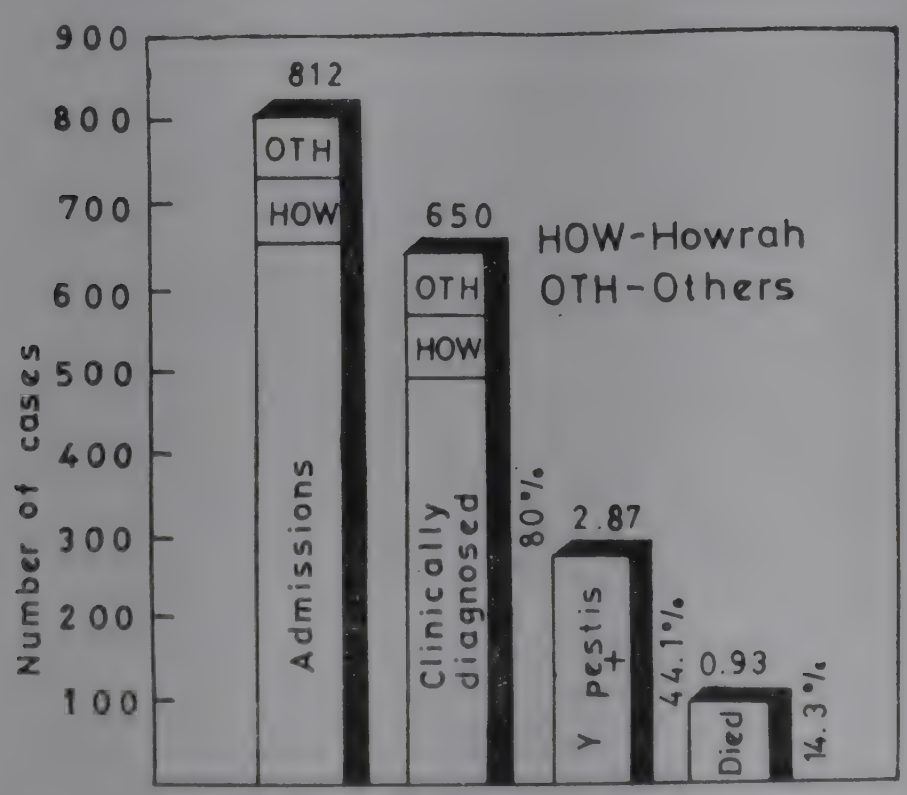

Fig. 13. Total admissions and plague cases detected and number bacteriologically confirmed during 1948-1952 in Calcutta and Howrah compared to that of 1898-1925.

Wardwise Distribution

At the time of this epidemic, Calcutta had only 32 wards, of which cases were reported from 27 wards in 1948 and 30 wards in 1949 (only two wards 17 and 30 were spared). Wards mainly affected were 4, 5, 7, 8, 11, 19, 26, 28 and 32. The reason for such rapid and widespread distribution was due to the wide dispersal of the rats from instinctive danger to life at the onset of the epizootic. In Howrah only 3 wards were affected in 1948 and 9 out of 10 wards in 1949. The geographical distribution has been shown in Fig. 22.

Seasonal Distribution of Cases

The combined seasonal distribution of plague cases for 1948-52 has been compared with that of the period 1898-1925 in Fig. 14.

The experiences of the two periods are similar except that in the long continued prevalence there was a left shift of the peak months to March and April instead of April and May for the aborter prevalence of the epidemics. In both the instances nearly 87 per cent of cases occurred during the four months of March to June.

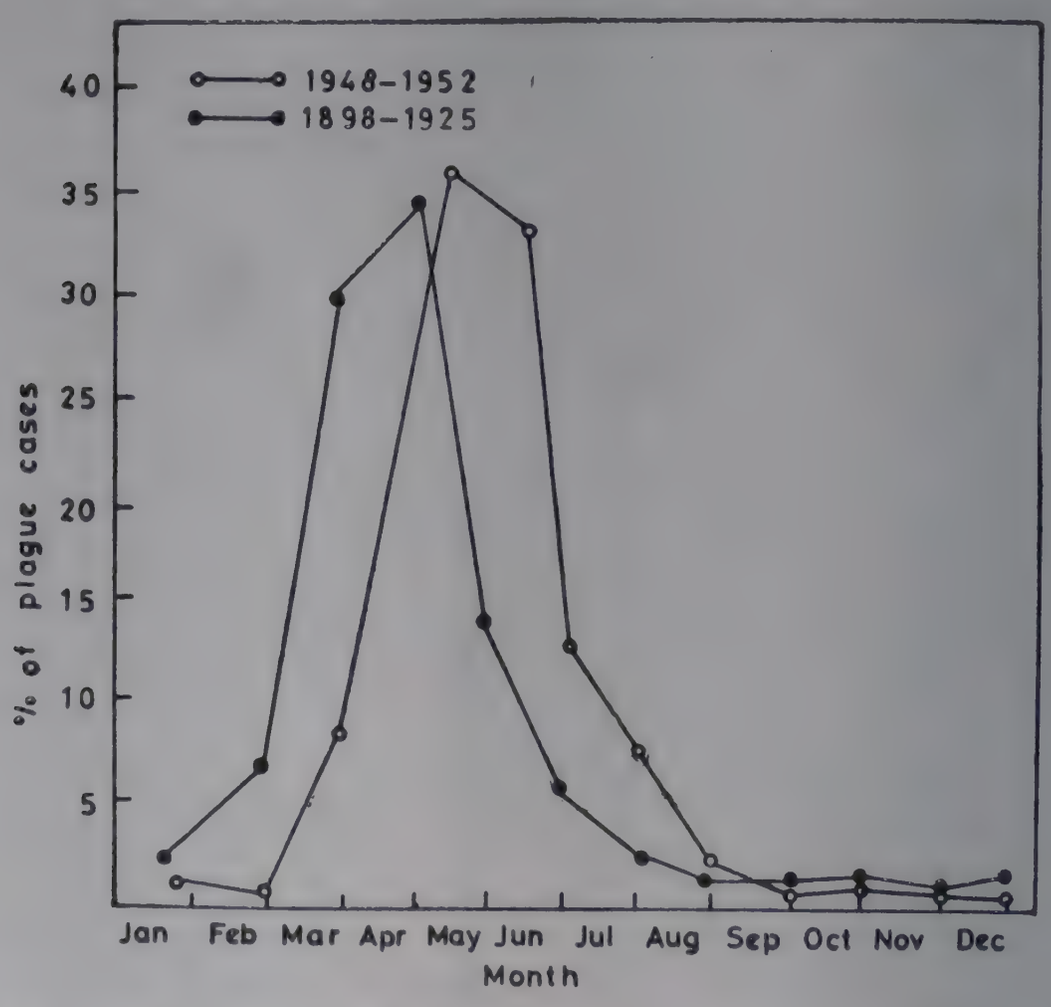

Fig. 14. Combined seasonal distribution of plague cases of 1948-52 compared to that of the period 1898-1925 in Calcutta.

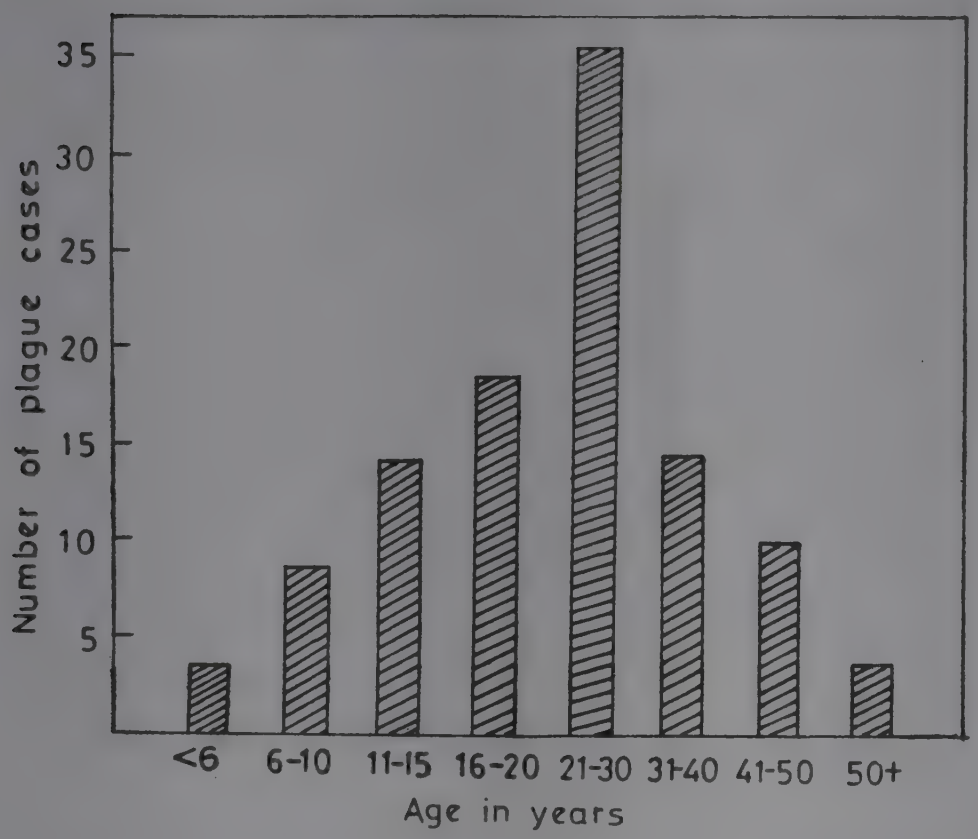

Fig. 15. Age incidence of plague cases in Calcutta and Howrah (1948-1952).

Age and Sex Distribution

The percentage distribution of plague cases in Calcutta by age during 1948-52 is given in Fig. 15. About half the cases belonged to the age group 21-40 yr, the peak being in the age group 21-30 yr, *i.e.*, the working group. Male : Female ratio was about 5 : 1 (82.7 against 17.3%). One reason of this distribution is that a large proportion of working population live in this city without their families and secondly females cover themselves more intensively than the males.

Multiple Cases in the Family

From a study of 245 affected families the distribution of plague was—single case in 177 families (72.2%); two cases in 61 families (25.0%); three cases in 4 families (1.6%); four cases in 2 families (0.8%); and five cases in 1 family (0.4%; Fig. 16).

Clinical Types

Out of 812 admission in hospitals and cases detected in the field in 1948-52 in Calcutta, clinical types were noted for 618 cases (1948-140; 1949-417; 1950-61) and shown in Fig. 17.

Distribution of Plague Cases by Religion

The distribution of plague cases by religion during 1948-52 was as follows: Hindus—70.0 per cent; Muslims—27.9 per cent; Christian—1.8 per cent; Others—0.3 per cent. Proportionately Muslims suffered more than the Hindus, others less than the representative proportion.

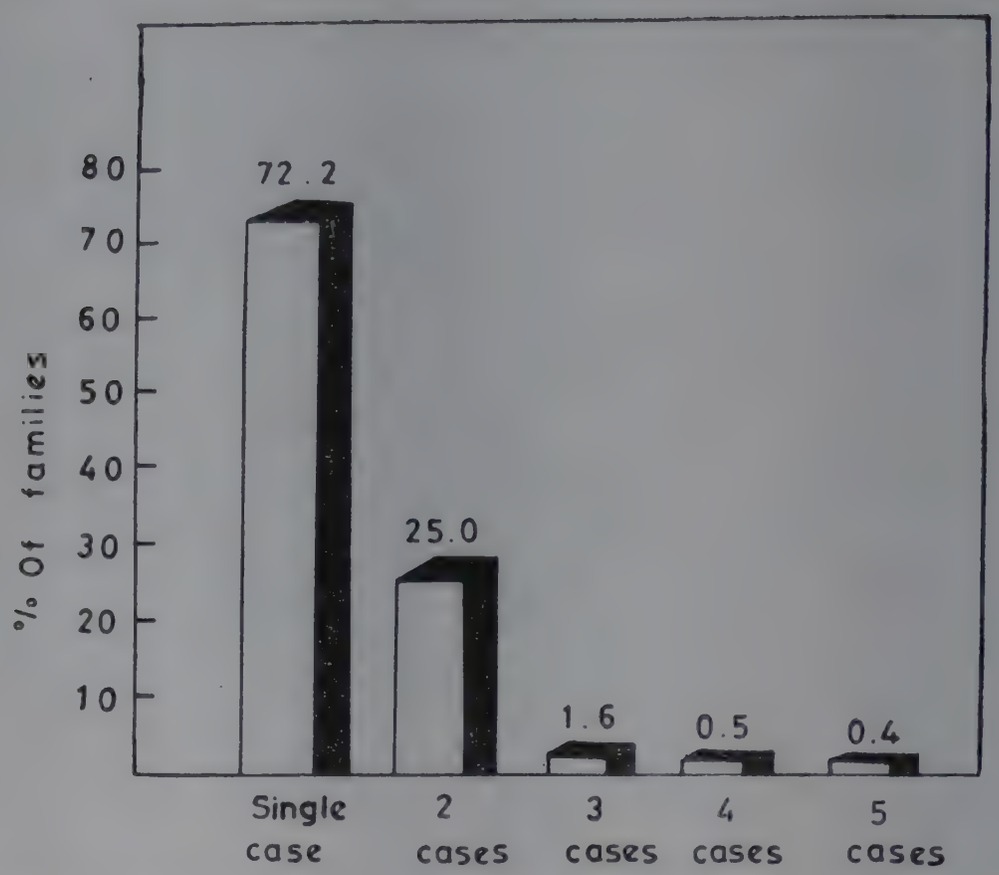

Fig. 16. Per cent distribution of families with single or multiple cases of plague in Calcutta and Howrah, 1948-49.

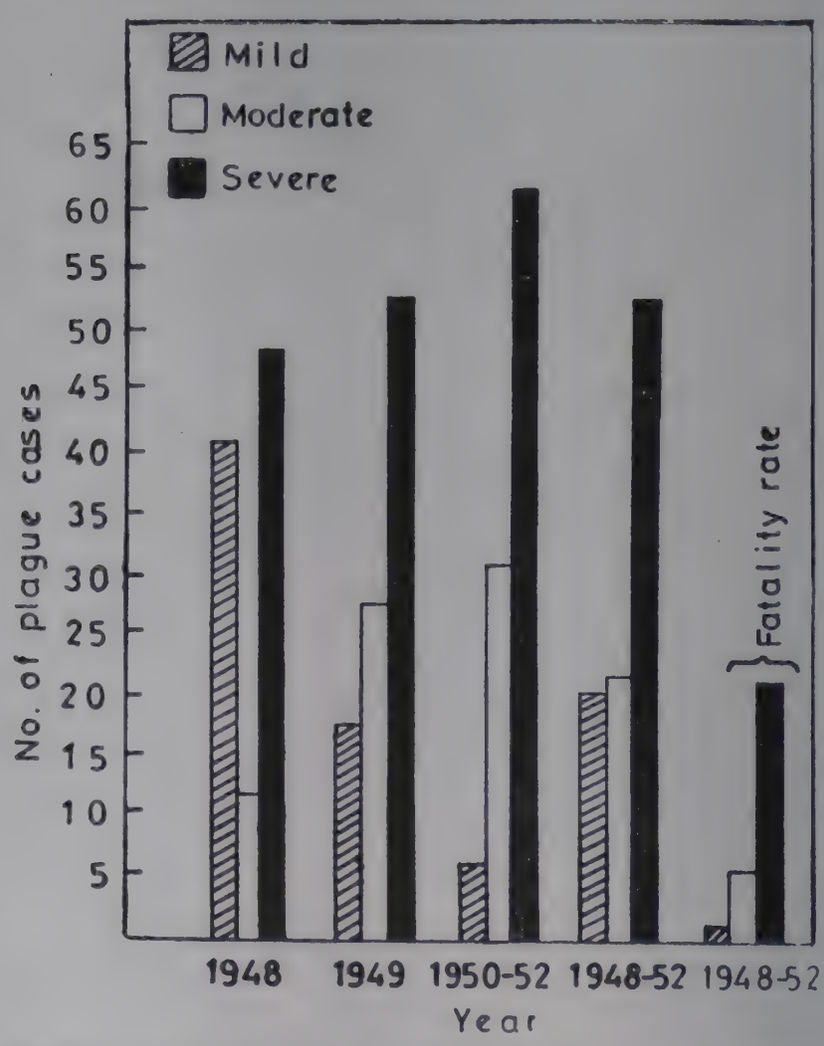

Fig. 17. Distribution of plague cases according to severity and fatality rates in Calcutta (1948-1952).

Types of Houses from which the Plague Cases were Reported

The percentage distribution of the nature of houses from which plague cases were hospitalised was: residential (85.3%), trading shop (4.2%), grocer's shop (0.6%), godown (4.2%), restaurant (0.3%), mess and hostels (0.3%), market (1.5%), stable (0.3%) and others (3.3%).

Rodent Densities in Calcutta during 1948-1950

A total of 38,241 cage traps were laid during the years 1948-50 in different wards of the city and 15,984 rats were caught (few rats had escaped). The average density was 41.7, varying from 8.5 in ward 15 to 81.8 in ward 32 (These wards were later reorganised into 100 wards and hence comparative densities by wards have lost their values). These densities were, however, not found related to human plague which depended on the relative distribution of the species of rodents and vector fleas. The density of *R. rattus* was below 1.0 in at least 8 wards and above 3.0 in 7 wards, the maximum being 13.4 in ward 4 and 8.5 in ward 8, both plague infected. In the remaining 15 wards it ranged between 1.0 and 3.0.

The rodent densities by months (1949-50) are given in Fig. 18 from which it will be seen that the highest density (133.7) occurred in April, the peak month for plague cases. There was a fall, fluctuating slightly from May to

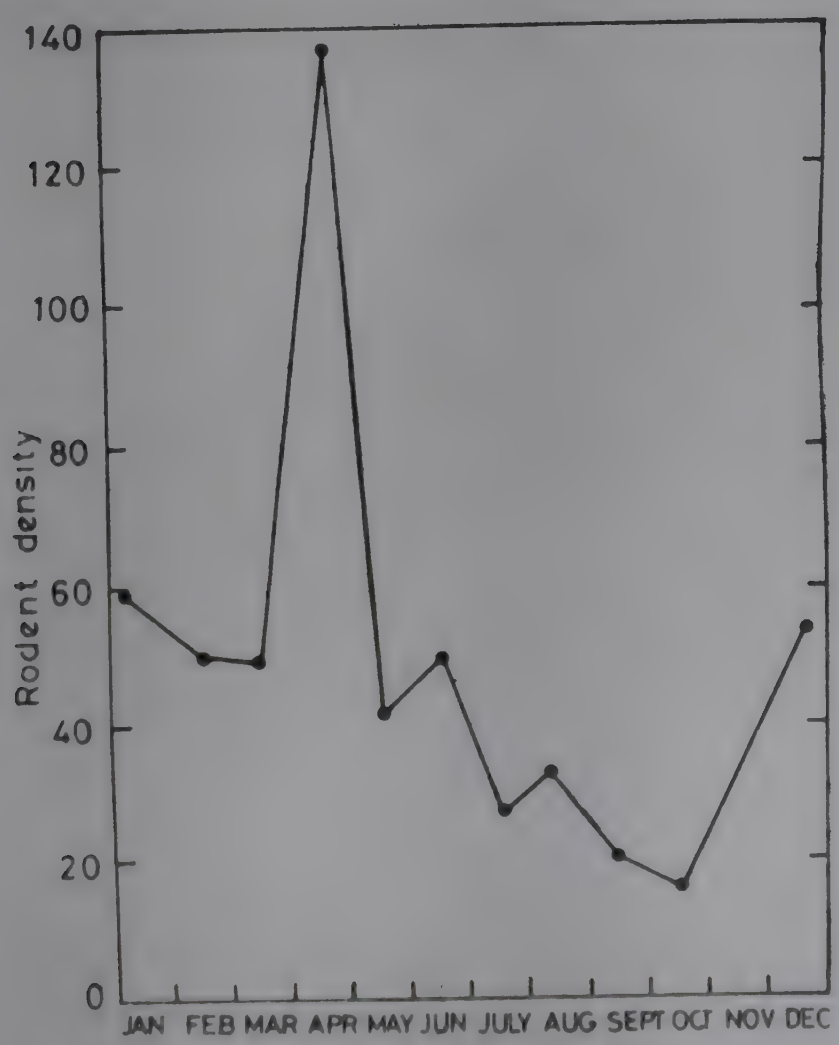

Fig. 18. Seasonal rodent density in Calcutta (1948-1956).

October (13.6) following which there was a rise and then almost a steady position around 50-58 per cent.

The percentage of the houses with rat harbourage was low in 10.7 per cent; moderate in 39.1 per cent and heavy in 43.3 per cent. 6.9 per cent houses did not harbour rats. The occupational status of the residents of these houses is given in Table 6. 1.3 per cent of these residents were rich, 1 per cent belonged to upper middle class, 4.6 per cent to middle class; 21.1 per cent to lower middle class and 65.9 per cent were poor. Beggers comprised 0.3 per cent of the affected plague cases whereas 5.8 per cent belonged to other sections of society. Thus it was observed that majority of plague cases was from poor and lower middle classes living in over crowded houses with rat harbourage.

Species of Rats in Calcutta between 1948 and 1956 Compared with those of Bombay and Madras

The data in Table 7 show that in Madras *R. rattus* was the predominant species until 1930, at which time, indeed, it was practically the only species present (No more recent data are available). In Calcutta the domestic species made up only 14.0 per cent of the total in 1906 and remained at almost the same level until 1950. Since the last epidemic, however, it suffered a reduction by about 50 per cent. *R. norvegicus*, the common known rat of Europe, was not heavily represented in Calcutta and fluctuated between 9.0 and 26.0 per cent during the last few years following the epidemic. The relative proportion of rats in Bombay had also undergone important changes. *R. rattus* declined from 66.2 per cent in 1910 to nearly a third of the proportion by 1946 and fluctuated between 23.7 and 22.9 per cent from this year and 1956. *R. norvegicus* declined from 28.7 to 15.9 per cent in 1956. The

Table 6. Occupation status of plague cases

Nature of occupation	No. of subjects
Liberal profession	3 (0.5)
Trader	13 (2.25)
Shopkeeper	36 (6.3)
Vendor	23 (4.0)
Clerk	5 (0.9)
Transport labour	13 (2.2)
Artisan	38 (6.6))
Industrial labour	60 (10.4)
Porter	81 (14.1)
School-going	57 (10.0)
At home	123 (21.4)
Sweeper	8 (1.4)
Others	115 (20.0)
Total	575 (100.0)

Figures in parentheses are the percentage values.

Table 7. Percentage distribution of different species of rodents in Calcutta, Bombay and Madras

City	Author	Year	*R. rattus*	*R. novergicus*	*B. bengalensis*	*B. indica*	Others
Calcutta	Hossack	1906	14.0	26.0	60.0	—	—
	Rao	1936	13.5	22.0	27.3	—	37.2
	Seal & Bhattacharjee	1948-50	13.3	9.0	75.8	1.7	—
	Chaudhuri (Personal Communication)	1951	6.1	17.3	75.8	0.75	—
		1952	6.5	24.3	68.1	1.1	—
		1953	6.1	26.1	66.1	1.2	—
		1954	5.6	23.8	69.8	0.8	—
		1955	5.2	17.2	73.1	0.5	—
		1956	7.0	9.0	79.9	0.3	—
Bombay	Indian Plague Commission	1910	66.2	28.7	1.0	—	4.1
	Webster & Chitre	1930	79.1	11.0	9.9	—	—
	MOH, Bombay	1946	23.7	18.3	47.4	—	10.0
	Haffkine Institute	1950	22.6	17.7	38.1	—	21.6
		1954	24.7	17.8	38.1	—	19.4
		1956	22.9	15.9	49.2	—	12.0
Madras	India Plague Communication	1910	49.4	—	—	—	50.6
	King and Pandit	1931	98.8	—	—	—	1.2

biggest change in Bombay had been in the proportion of *B. bengalensis* (Gunomys kok) which constituted only 1 per cent of the total in 1910 and had risen to 47-49 per cent between 1940 and 1956. This rodent being fairly susceptible, the city was considered by some workers to be vulnerable to plague infection and human cases were actually reported in the city in 1948, 1949 and 1952. Fortunately, *R. rattus* and *R. norvegicus* were still very resistant to plague.

Rat Falls and Types of Rats Involved

The types of rats involved were *R. rattus*, *R. norvegicus* and *Gunomys kok* (*B. bengalensis*). Out of 891 rats examined between 20th April 1948 and May 1948, 19 or 2.1 per cent showed evidence of plague infection. The largest number of dead rats were from ward 8 where from the plague cases actually had started and the rats were found in plague infected location in 9 other wards up to a distance of 8 to 10 km, such as, Dum Dum, indicating quick spread of infection through rat movement.

After the plague laboratory was established in June 1949, 75,187 rodents including mice and shrews, living or dead (7180) from Calcutta and 3880 from Howrah were collected. Out of these 23611 rodents were identified, their proportional distribution was as follows: *Gunomys kok*—82.5 per cent; *R. rattus*—5.6 per cent, *R. norvegicus*—11.4 per cent and *Bandicota indica*—0.7 per cent.

Dissection of Rats

Of the 23611 rats identified 13596 were dissected for detection of plague infection. Their proportional numbers were: *Gunomys kok*—69.27 per cent; *R. rattus*—19.8 per cent; *R. norvegicus*—11.2 per cent; and *Bandicota indica*—0.7 per cent (Fig. 19). After dissection smears were made from spleen, liver

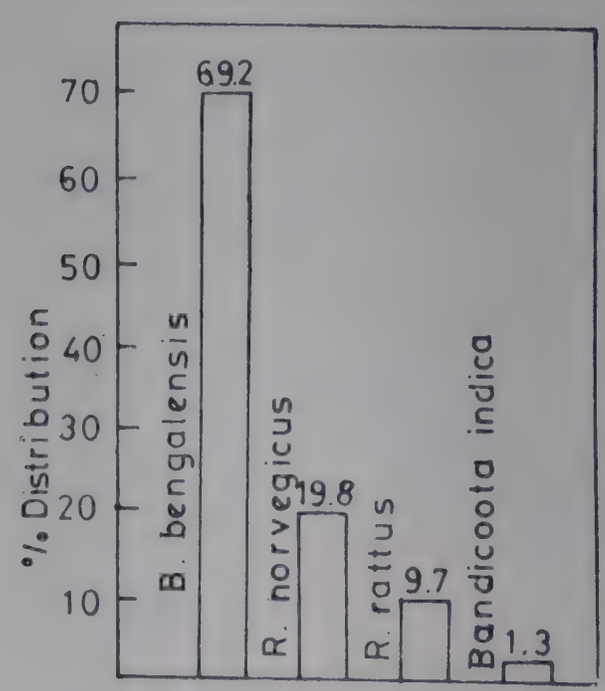

Fig. 19. Per cent distribution of rats in Calcutta and Howrah (1948-1952).

and enlarged glands and examined microscopically for *Y. pestis* and the organism recovered by culture was tested for virulence in mice. The number of organisms causing 80 per cent deaths of the experimental mice indicated the degree of virulence of the organism.

Human Cases and Rodent Infection

During the years 1948 to 1960, 612 (94.2%) cases out of 650 were diagnosed as bubonic and 22 (3.4%) as septicaemic, and 16 (2.4%) as pneumonic. Of these 93 died giving a fatality rate of 14.3 per cent. The outstation cases were 12 in 1948 and 30 in 1949 involving 10 adjacent municipalities and towns.

In 1960, rat falls were reported from three places in rural West Bengal namely, (*i*) Murarai in the district of Birbhum, (*ii*) Contai in the district of Midnapore, (*iii*) Golsi in the district of Burdwan. On investigation only one bubonic case was detected in Murarai where out of 55 rats examined bacteriologically *Y. pestis* was isolated in three instances. This place was revisited in 1971 to investigate the possible persistance of plague infection among the local rats. But no plague infection was detected in any of the 90 rats examined. Serological test carried out by gel-precipitation test of Seal (1972) indicated the presence of specific antibody against plague in 22 rats (24.4%).

The number of infected rodents detected in Calcutta and Howrah during the period between 1948 and 1960 is given in Table 8. This Table shows that during the period of observation, out of 113 individually positive rats 36 (31.86%) were *R. rattus*, 38 (33.6%) *R. norvegicus*, 35 (31.0%) *B. bengalensis* and 4 (3.54%) *B. indica*. Besides, out of 97 pooled groups of rats examined three groups consisting of mixed type of rats yielded positive isolation. All the isolations were subjected to virulence test in white mice and were found more or less virulent except a single strain in 1971 which was avirulent. Taking one rat as positive in the three groups the total number of rats found positive among 1138276 rats examined during 1948 to 1960, was 116 (0.01%), while a single rat was found positive of avirulent strain among 737523 rats examined between 1961 to 1971. No human case was reported in Calcutta and Howrah after 1952 and interestingly no *R. rattus* was found bacteriologically positive after 1953 although positive isolations could be made among *R. norvegicus* and *B. bengalensis* till 1958. *B. indica* was very few yet positive isolation was made till 1953.

Resistance of Local Rat Population

A total of 374 rats, 114 from ward 8 (affected) and 95 from ward 10 (plague-free), 85 from ward 25 (affected in 1949) and 80 from ward 22 (affected in 1949) were tested for residual resistance to plague in 1953-54 with an infective dose of 5000 virulent plague bacilli. In some subsequent experiments 2500, 500 and even 100 organisms were used without affecting the result by delaying death for a few days in the susceptible rats. In ward 8,

Table 8. Number of infected rodents detected in Calcutta and Howrah between 1948 and 1971

Year	No. of rates dissected	No. found infected				Total
		Rr.	Rn	Bb	Bi	
1948	891	7	12	—	—	19
1949	17938	12	8	10	1	31
1950	43557	3	—	11	1	15
1951	89889	4	4	2	—	10
1952	59021	7	7	2	1	17
1953	63620	3	—	1	1	5
1954	87639*	+	+	+	—	3*
1955	136645	—	3	—	—	3
1956	145846	—	1	—	—	1
1957	130393	—	—	5	—	5
1958	113993	—	1	2	—	3
1959	103295	—	—	—	—	—
1960	84669 80 (Murarai)	—	2	1	—	3
1961	737523	—	—	1**	—	113 (+3)

*Out of 97 groups of pooled rats examined three groups of 20 rats were each of mixed type. **The strain isolated was avirulent.

the original focus of plague infection, 16 out of 114 rats survived (14%). This included *R. rattus*—40 per cent, *R. norvegicus*—7.2 per cent, *B. bengalensis*—1.8 per cent indicating *R. rattus* having the major share of the epizootic was still maintaining some infection giving a higher proportion of resistant rats than the other two. On the other hand, all the three types of rats in ward 10 which remained plague-free all through showed 100 per cent susceptibility. In wards 22 and 25 affected in the earlier part of the outbreak, *R. rattus* was only partially resistant with 19 per cent surviving, and the other rats were highly susceptible. In Bombay, on the other hand, *R. rattus* and *R. norvegicus* were found highly resistant between 1952 and 1956, while *B. bengalensis* was highly susceptible (Table 9). Madras rats were fully susceptible according to the results obtained by the Haffkine Institute (Habbu, 1960).

Examination of Fleas

A regular examination of fleas in 1948 collected from traps, covers and body of the rats (died or killed in cyanogas chamber) for general and specific indices gave results shown in Table 10.

It appears that in 1948 the specific flea index was very high both of cheopis and astia in the early part of the epizootic in contrast with Rao's observation during the non-epidemic year 1936. The indices, however, came down

Table 9. Percentage mortality among Bombay rats in resistance test conducted at the Haffkine Institute compared with Calcutta data

Species	Bombay rats					Calcutta
	1952	1953	1954	1955	1956	1953-56
R. rattus	12.6	15.7	12.1	16.0	7.6	81.0
R. norvegicus	—	5.8	3.3	2.6	2.2	94.7
B. bengalensis	77.6	82.5	76.7	70.0	75.2	97.6

Table 10. Indices by species of rats and season in 1948 as compared with 1936

Species	April-May, 1948			June-Sept. 1948			Rao, 1936*		
	Cheopis	Astia	Gen.	Cheopis	Astia	Gen.	Cheopis	Astia	Gen.
R. rattus	4.91	4.84	9.75	0.68	0.78	1.46	3.0	1.3	4.3
R. norvegicus	2.63	2.96	5.59	0.68	2.08	2.76	3.3	6.1	9.4
B. bengalensis	0.23	0.99	1.22	0.68	1.11	1.79	1.4	3.7	5.1
B. indica	0.60	0.83	1.49	0.0	0.06	0.05	0.7	3.3	4.0

*Non-epidemic year; Gen. = general.

in June-September following intensive DDT drive and cyanogassing of burrows along with the waning of the epizootic. Also fleas collected and examined from 20 rat burrows in June yielded organism resembling *Y. pestis* but serologically negative and biochemically also did not conform to be *Y. pestis*. Further investigation was abandoned.

In 1949, the peak year of the epidemic, out of 48530 rats (dead or alive) of a total collection of 75187 rats, 43403 fleas were collected. The total and specific flea indices month by month are given in Table 11.

Table 11 shows that at the beginning of 1949 the flea indices were fairly high though the *cheopis* index was below 1.0 (0.70) during the first three months of the year Plague began to rise from February and that the rat and flea control programme could be augmented only after the plague control laboratory was established in June, 1949. It was seen that the control operations were effective in bringing down the *cheopis* index to 0.04 by December relatively increasing the *astia* index 1.24 in December.

Flea Species and Distribution

Table 12 shows that the predominant flea in Calcutta and Bombay were *Xenopsylla astia* (65.6%) and *X. cheopis* (76.3%) respectively. In Madras *X. astia* was practically the only flea of importance (95.3%), but recent figures are not available. The percentage of *X. astia* in Calcutta seems to have

Table 11. Seasonal distribution of total and specific flea induces in 1949

Month	Total flea	Specific flea indices	
		X. cheopis	*X. astia*
January	2.59	0.72	1.87
February	2.4	0.67	1.73
March	2.05	0.70	1.35
April	1.24	0.33	0.91
May	1.35	0.32	1.03
June	0.63	0.19	1.44
July	0.67	0.11	0.56
August	0.61	0.05	0.56
September	0.68	0.06	0.62
October	0.92	0.06	0.86
November	0.85	0.05	0.80
December	1.25	0.04	1.24
Total flea collected	43,408	6,612	36,791

Table 12. Percentage distribution of different flea species in Calcutta, Bombay and Madras

City	Investigator	Year	*X. cheopis*	*X. astia*	*X. brasiliensis*	Other
Calcutta	Strickland and Roy	1925-26	40.0	60.0	—	—
	Rao	1936; 1941	40.4	59.6	—	—
	Seal and Bhattacharji	1948-50	34.4	65.6	—	—
Bombay	Cragg	1920	49.5	49.8	0.7	—
	Cragg	1923	53.1	45.8	0.1	—
	Webster	1928	77.5	19.2	3.1	—
	Webster and Chitre	1930	69.6	27.9	3.3	—
	Deoras and Tampi	1956	76.3	23.7	0.0	—
Madras	King and Pandit	1931	5.6	94.3	1 flea	1 flea

been increasing since 1925 at the expense of *X. cheopis*. Bombay, on the other hand in 1920 had almost equal proportions of these two fleas, the trend towards predominance of *X. cheopis* becoming significant in 1928 and being maintained thereafter; *X. brasiliensis* which was found there in low percentage, has now practically disappeared. It may be mentioned here that the Madras City has almost always been entirely free from plague.

Flea Indices

According to P. Chaudhuri (personal communication, 1958), who has been carrying out flea and rat control measures in the city for the past six or seven years, the general flea and the *X. cheopis* indices have been low since 1948, and more particularly during the following few years. An interesting point is that plague was raging in Calcutta during 1948-50, even when the average *X. cheopis* index was as low as 0.4. However, during the 1949 epidemic both *cheopis* and *astia* indices (Table 13) and the *R. rattus* index (Table 14) had increased in the focal plague areas during the rise of the epidemic and a noticeable difference was found between the plague-affected and the plague-free wards. Another point of interest is that both the general and *X. cheopis* indices were very low in 1925-26, when plague ceased in Calcutta after the first epidemic. These indices have now been still further reduced, and explain the current absence of human plague in the city in spite of the fact that occasional infection of rats was detected for a few years following the cessation of the epidemic. It has also been observed that both the general and the *X. cheopis* indices are usually highest in March-April and lowest in October-November although in 1957, for instance, the situation was reversed as regards the general flea index through the influence of *X. astia.*

For Bombay recent data also show much reduction in the flea indices—1.58 in 1953 as against 6.3 in 1930.

There are no recent figures for Madras, but the *X. cheopis* index was very low there in 1931 and this may be one of the reasons for the absence of plague in that city.

From the data given in Table 14, it may be concluded that in plague-affected cities like Bombay and Calcutta, *R. rattus* and *R. norvegicus* are better host to *X. cheopis* than *B. bengalensis* though *B. bengalensis* carry more *X. astia* than *X. cheopis* in Calcutta but not in Bombay. The danger of plague is clearly much greater from the two *rattus* species than from the bandicoot. This is corroborated by our earlier findings during the last outbreak at Calcutta. Out of a total of 50 rats found bacteriologically plague infected the percentage distribution was: *R. rattus*—46 per cent; *R. norvegicus*—34 per cent; *B. bengalensis*—18 per cent; others—18 per cent. The first two together consitute 80 per cent of the total positives.

Examination of Fleas for Plague Infection

In experiments conducted in wards 8 and 10 of Calcutta in 1953-54 fleas from each rat cage were pooled and crushed for mouse inoculation and culture. Except for one pool from ward 8 in March, 1954, all samples proved to be negative. There were no human cases in 1953 or 1954 but the rats were found positive on several occasions (Chaudhuri, P., personal communication).

The relative proportion of two species of fleas differed in the two wards, being 112 *X. astia* per 100, *X. cheopis* in ward 8 and 277 *X. astia* per 100 *X. cheopis* in ward 10. The average daily number collected fell to a

Table 13. General and specific flea indices in Calcutta, Bombay and Madras

Author	Year	General flea index	*X. cheopis* index	*X. astia* index	*X. brasiliensis* index
Calcutta					
Strickland and Roy	1925-26	0.60	0.16	0.4	—
Rao	1936	5.70	2.30	3.40	—
Seal and Bhattacharji	1948-50	1.10	0.40	0.70	—
Chaudhuri	1951	0.56	0.12	0.44	—
	Minimum	0.27 Oct.	0.03 Nov.	0.23 June	
	Maximum	1.01 April	0.31 April	0.71 March	
	1954	0.84	0.19	0.65	
	Minimum	0.51 June	0.06 Nov.	0.46 Nov.	
	Maximum	1.63 March	0.58 March	1.25 March	
	1957	0.40	0.05	0.35	
	Minimum	0.28 Jan.	0.01 Jan.	0.21 Jan.	
	Maximum	1.29 Oct.	0.17 Oct.	1.12 Oct.	
Bombay					
Webster and Chitre	1930	6.30	3.10	3.10	0.10
Deoras and Tampi	1956	1.58	1.20	0.37	0.90
Madras					
King and Pandit	1931	2.60	0.1	2.5	1 flea

Unpublished data.

Table 14. Flea indices according to rat species in Calcutta, Bombay and Madras

Author	Year	*R. rattus*		*R. norvegicus*		*B. bengalensis*	
		X. cheopis index	*X. astia* index	*X. cheopis* index	*X. astia* index	*X. cheopis* index	*X. astia* index
Calcutta							
Strickland and Roy	1925-26	low	low	0.85	2.51	1.17	2.35
Rao	1936	3.0	1.3	3.3	6.1	1.4	3.7
Seal and Bhattacharji	1948-50	0.8	0.6	0.9	1.0	0.2	0.7
Bombay							
Webster and Chitre	1930	3.7	0.5	4.4	2.5	9.2	2.7
Deoras and Tampi	1956	1.44	0.17	—	—	0.9	0.63
Madras							
King and Pandit	1931	0.2	3.7	—	—	—	—

comparatively low figure between July and November and also in February. It was high in December and again in March-June, with the highest peak of collection in March. Thus laboratory observations support those made in the field.

Relationship of Flea Indices to Plague Cases

The *X. cheopis* index in 1949 was found to be the highest in Calcutta during January-March and lowest in October-December, while the percentages of rats positive and of human cases were highest in April-June and lowest in October-December (Table 15). It appears that the *X. cheopis* index remains high from the end of March to April, when the rat epizootic starts. The number of human cases builds up about two weeks later, which explains the higher percentage being found in the April-June period. Closer analysis, however, shows that there is a distinct relationship between the *X. cheopis* index and the epizootics as well as the human epidemic.

Although *B. bengalensis* have been found infected with plague and although chronic plague has also been found frequently among them in Calcutta, it appears from the above data that among the rodents *R. rattus* and among the fleas *X. cheopis* are two important elements (reservoir plus vector) in urban human plague infection in India. If either of them can be kept down, the risk of human plague can be reduced to a minimum. *R. rattus* abounds in *bustees* (slum areas) and houses with tiled or thatched roofs and in godowns and mills.

The city which can completely do away with houses of these kinds and which can properly control conditions in godowns and mills can perhaps eliminate *R. rattus*. Well-built brick houses with rat-proofing devices offer no shelter to *R rattus*. Wherever such buildings are found in large numbers, *R. rattus* is scanty or absent, and human plague is not seen there. The *R. rattus* density, however, is fairly high in ward 8 in Calcutta, and it is there that plague has always been found to start.

Another way to control urban plague would be to keep the *X. cheopis* index at a negligible level or to attempt to eradicate the species altogether.

Pneumonic (Interhuman) Plague

Pneumonic plague is the deadliest of all diseases. The essential facts about

Table 15. Relationship between flea indices, plague cases and rat infection in Calcutta by trimestrial period of 1949

Period	*X. cheopis* index	*X. astia* index	Total flea index	% of infected rats	% of plague cases
January-March	1.0	0.8	1.8	38.0	12.5
April-June	0.6	0.6	1.2	54.0	81.0
July-September	0.6	1.2	1.8	2.0	5.5
October-December	0.1	0.6	0.7	6.0	1.0

this form of plague were first assembled by Wu Lien Teh (1926) in his monograph on 'Pneumonic Plague'. Many interesting observations on this subject are also contained in a report on plague in Egypt by Petrie *et al* (1923) and in a series of papers by Girard (1927). Mention had also been made of the localised outbreak in South Africa, California, South-East Russia and Argentina in areas reporting wild-rodent plague. It usually originated from bubonic plague complicated with plague pneumonia perhaps following septicaemia. The first clear clinical description of infectious interhuman pneumonic plague was made by Childe (1897) as it sometimes occurred in association with bubonic plague in Bombay in 1896-97. Interestingly symptoms of bronchopneumonia generally do not commensurate with the severity of infection. Almost the first indication besides high fever may be slight cough and bloody sputum. In India, primary pneumonic plague occurred only in circumscribed areas seldom spreading beyond families first affected. The author had the opportunity of studying two instances of pneumonic plague outbreak, one at Calcutta in 1948 and another at Gaya in 1949 both being associated with an outbreak of bubonic plague (Seal, 1949; Seal and Prasad, 1959).

In the earlier phase of the last pandemic pneumonic cases (all fatal) were also reported from Calcutta and Eastern Bengal (now in Bangladesh) but the instances were few and far between as will be seen from the following records (Tables 16 and 17) collected by Crake (1908).

During this pandemic, besides the instances mentioned above, small outbreaks of pneumonic plague were occasionally reported from other areas in India, namely, Bombay, Baroda, Cutch, Karachi, Kankhal, Jaswalpur, Madras, Punjab, N.W.F.P. and Kashmir. Barring a few exceptions pneumonic plague caused little havoc in India in the last pandemic. The biggest outbreak of pneumonic cases was, however, in Manchuria in 1910-11 which led to the establishment of the International Plague Conference at Mukden in

Table 16. Pneumonic plague cases in Calcutta (1898-1907)

Year	Pneumonic cases	Percentage of total cases	Remarks
1898	9*	4.7	11* cases also occurred at Buckerganj among contacts
1899	116	13.4	
1903	235	3.1	
1904	101	2.1	
1905	**	3.7-7.5	In one district only
1906	190	6.5	
1907	219	5.5	

*These cases were from one house.
**The number of cases not mentioned in Crake's report.

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Table 17. Pneumonic plague cases in Eastern Bengal

Year	Month	Locality	Cases	Deaths	Remarks
1898	September	Buckerganj	11	11	Introduced from Calcutta
1899	(i) Feb-March	Pania Village	42	38	-do-
	(ii) March	Faridpur	30	29	Two bubonic cases preceded the pneumonic cases
1906	(i) March-April	Serajganj	27	24	Introduced from Calcutta
	(ii) June	Mymensing	5	5	-do-
	(iii) August	Mymensing	50	45	-do-
1907	January	Maldah	4	4	Introduced from Balia, Behar
1910	June	Noakhali	51	45	Introduced from Calcutta
1911	June	Faridpur	17	17	-do-
			237	218	(92.0%)

1911. According to the Indian Plague Commission (1908) pneumonic plague cases were rare not exceeding 3 per cent of all cases. In the evolution of plague epidemic, therefore, the character of the parasite (plague bacillus) particularly with reference to occasional changes into virulent and pneumonic form is of the greatest importance but the mechanism of this sudden change remained a mystery.

Pneumonic plague is generally a disease of the cold climate and is more liable to break out in winter than in summer. The Indian outbreaks mentioned above also occurred during the cold season. In other places it occurred in sporadic form and also in the moist and warm climate of Java, Madagascar, Ceylon (Sri Lanka) and in the dry and hot climate of Egypt, but all in sporadic form. Perhaps certain temperature above 90-95°F is not conducive for man to man propagation of plague bacilli through heated air.

Epidemiologic Features of Pneumonic (Interhuman) Plague

(*a*) *Seasonal and Climatic Variations vis-a-vis Bubonic Plague*

In bubonic plague the optimum conditions of temperature and humidity are approximately represented by a mean temperature of 68°-77°F in association with a relative humidity of the order of 60 per cent and above. There is a marked decrease in the incidence of plague if the mean temperature rises above 90°F. The largest number of cases occurs at times of high relative humidity. Pneumonic cases were generally interspersed with bubonic and never exceeded 3 per cent of the total in the present century epidemics. The seasonal incidence of pneumonic cases as seen in Calcutta in the period 1904-07 is shown in Fig. 20.

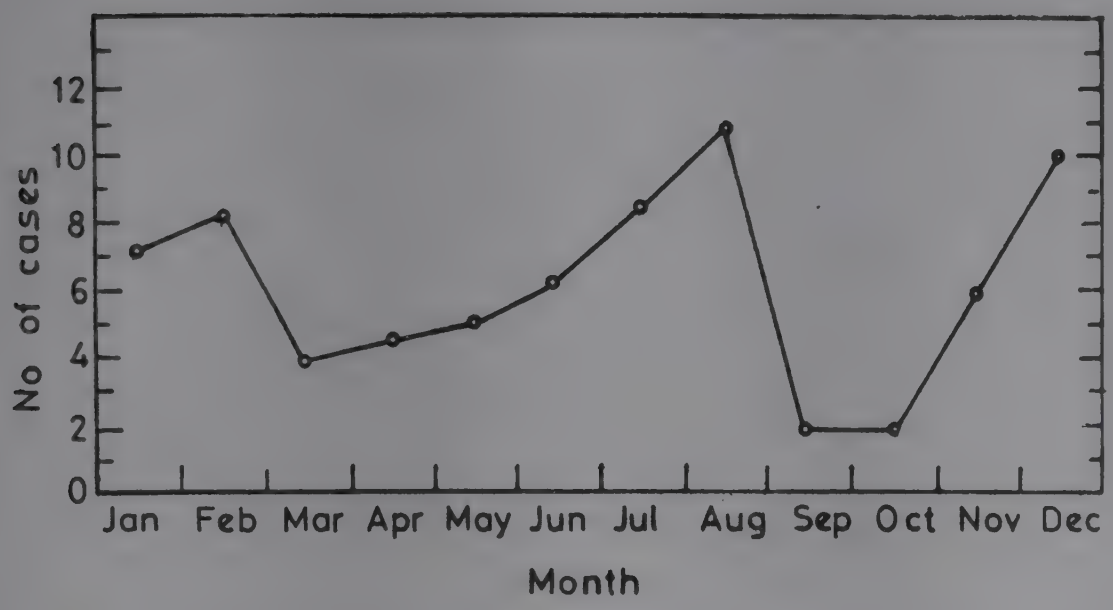

Fig. 20. Seasonal incidence of pneumonic plague in Calcutta (1904-1907).

(*b*) *How do Pueumonic Cases Arise*?

The organism isolated from pneumonic cases could not be distinguished from those of bubonic cases. The production of entirely distinct clinical types of disease by seemingly identical strain of the same organism is not however a peculiarity of the plague bacillus alone. It is well known that influenza may appear in different clinical forms *e.g.*, respiratory, gastro-intestinal and pertaining to CN system *etc.* Similarly there is difference between flea-borne and louse-borne typhus manifestations and between respiratory, intestinal, meningeal, glandular and other cutaneous forms of tuberculosis. Various explanations have been offered for this. Firstly, it is commonly associated with wild rodents which are more susceptible to lung infection (pneumotropism) than the domestic or peridomestic rodents. Petrie *et al* (1923) suggested racial susceptibility. Others proposed differential susceptibility of man under weather condition, overcrowding or a closed atmosphere during winter months. Strong and Treague (1912) pointed out that far more plague bacilli are disseminated into the air by sneezing and coughing than by speaking, spreading droplets teeming with bacilli into the upper respiratory tracts and lungs of contacts within certain range. Teh and Jettner (1921) sprayed *tarabagan* and *suslik* with a fine emulsion of hepatized lung containing masses of plague bacilli and found that every animal succumbed to plague mainly of primary pneumonic type, whereas the common laboratory animals like guineapigs, rabbits and rats were not easily infected experimentally by inhalation, but monkeys become an easy victim.

Girard (1929) believed that a mixed infection with pneumococci or influenza prepared the ground for pneumonic plague but otherwise fully healthy persons imbibed the infection by mere contact with pneumonic plague cases and died within a short time. Difference in the virulence of the organism was also suggested but ruled out by Wu Lien-Teh. Thus no satisfactory explanation has yet been found to fully explain the origin of pneumonic

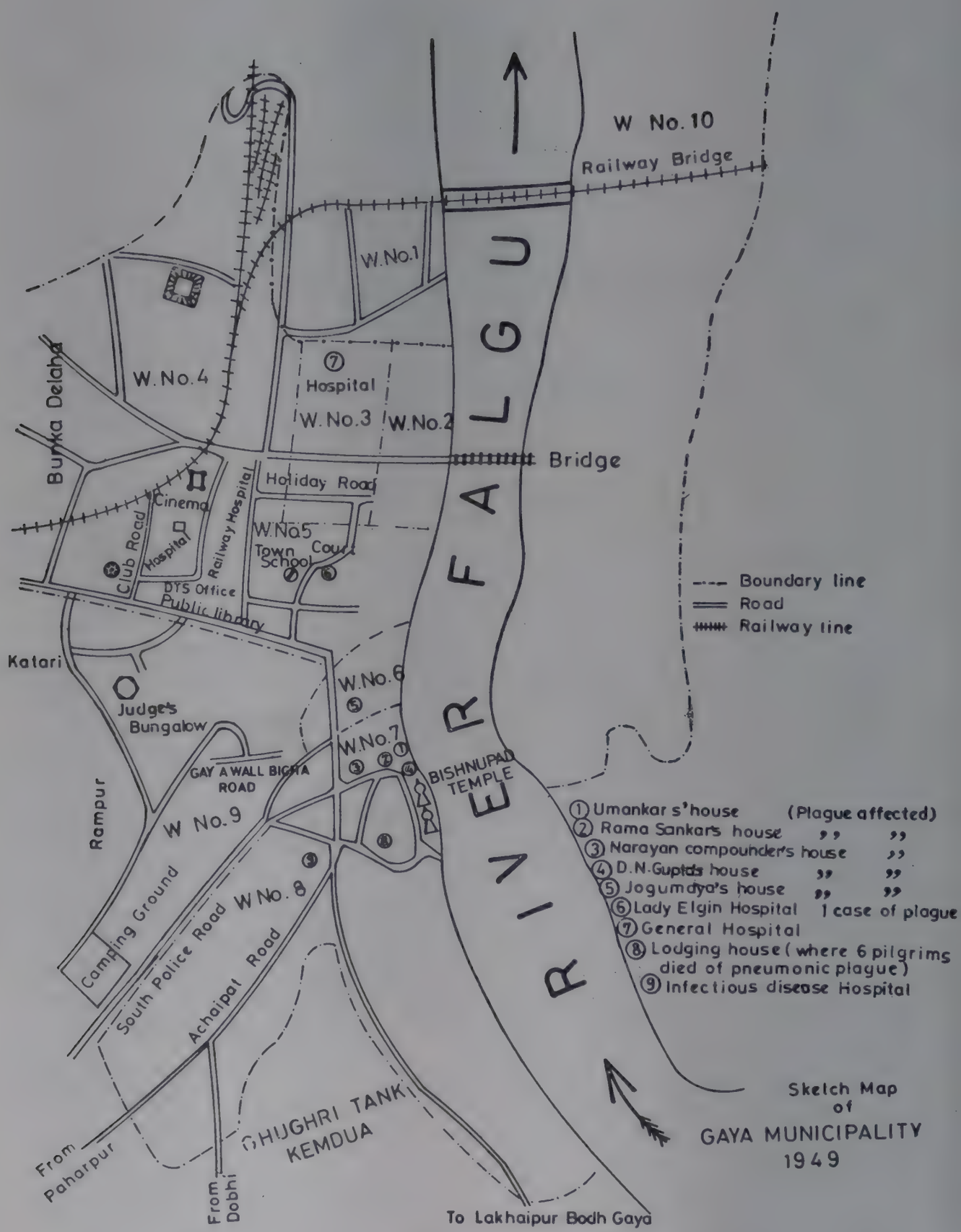

Fig. 21. Spot map of plague cases in Gaya Municipality, 1949.

plague. The author, however, noted that when the bubonic cases in the incubation period had prolonged journey and exhaustion, they got their lung involved before death and those attending such cases developed pneumonic plague and died. Contact and convalescent carriers were never discovered in pneumonic plague epidemics but interestingly the epidemic came down by itself (probably by exhaustion of contacts) although there was no evidence of immunity being developed in the population at risk as happens with other types of epidemic diseases.

Workers who had experience of epidemics of pneumonic plague believed that pneumonic plague was one of the easiest diseases to control if precautions are taken at the onset *e.g.*, complete isolation of active and suspicious cases. Surveillance operation with setting up of an emergency observation ward has to be established along with administration of prophylactic dose of antibiotics or sulpha drugs. Patients and suspects should be handled under strict coverage of masks and gowns to protect against the entry of infection by inhalation by persons duly inoculated by antiplague vaccine. Antirat measures by cyanogassing and other methods should also be simultaneously adopted to take care of the bubonic cases which generally precede pneumonic cases.

Distribution of Pneumonic Plague in Calcutta and Gaya

In Calcutta: while bubonic plague was raging in the city during 1949 *i.e.*, in the second year of its reappearance after 25 yr, a familial incidence of pneumonic plague occurred in a village (Bankul) 16 miles away from Calcutta involving 14 cases with 13 deaths. The origin of this outbreak was traced to a young shopkeeper of Kidderpore (Calcutta) who with fever (later proved to be due to plague infection acquired in Calcutta) moved to his village home. Apparently the exhaustion of the journey with plague infection led to plague pneumonia followed by 13 secondary cases by the time it was diagnosed as pneumonic plague (Seal, 1949).

Gaya: A total of 19 cases of pneumonic plague with 18 deaths was reported in the town of Gaya (Bihar), a perpetual pilgrim centre, in December-January (1948-49). It was traced to a pilgrim leaving the village Sail in Himachal Pradesh for Gaya in company with other pilgrims. He died of fever and pneumonia on the way apparently due to plague infection under circumstances similar to that of the Calcutta case. Six of the pilgrims died after reaching Gaya with the same type of disease followed by 13 more cases (Fig. 21) from three families starting with that of the physician who attended the cases. Diagnosis was confirmed by postmortem examination by a pathologist (Seal and Prasad, 1949).

Evolution of Knowledge on Plague Leading to its Ultimate Conquest

The chronological evolution of knowledge on plague over the centuries is described in the following pages. This evolution can be conveniently described as pertaining to five chronological phases. Phases I and II pertain to the ancient and mediaeval periods whilst phase III refers to the beginning of modern research on the source and transmission of plague, phase IV to the discovery of *Y. pestis* and transmission of plague and phase V to the advances in the knowledge gained during the mid-twentieth century.

CHAPTER III

Phases I and II of Evolution of Knowledge on Plague during the Primitive and Mediaeval Periods

In the natural history of diseases, most of the diseases which are microbial in origin came from the animal kingdom that preceded man in the chain of evolution and are at present prevalent among human beings in one of the following three forms *viz.*, (i) completely humanised, the animals becoming fully insusceptible typhoid, cholera *etc.*; (ii) affects both man and animal *e.g.* tuberculosis, leishmaniasis, rabies *etc.*; (iii) principally affecting animals and casually man. Plague belongs to the last category. In all the three categories animals can act as direct or indirect reservoirs or carriers. In plague however, man to man infection can also occur when it prevails among them in *pneumonic* form. Epidemics and pandemics created severe havocs in the earlier episodes mainly through the pneumonic form due to the absence of any scientific knowledge of its causative agent as well as of its mode of spread. Many theories and concepts were put forward about the nature of this disease, depending on the stage or progress of development of civilization and concurrent knowledge about the natural phenomena till the modern age. These are chronologically described here.

Supernatural Beliefs

Primitive man attributed all objects of the universe to animism. It was thought that neglected ancestral shades or offended spirits animating some venerated object were responsible for all afflictions including pestilence and disease. Accordingly, plague, like all other pestilences, was considered as the work of a demon, *Namtar*, who could be exorcised by incantations or his power neutralised by magic by the tribal sorcerer, who was the physician of the tribe. During plague season in Egypt, evil spirits were said to prick people with a magic lance poisoned by *Lucifer* (*Sticker, 1910a*). In an account of Byzantine plague epidemic of 542 A.D. Procopius mentioned about spectres in human shape to many who fell ill of plague. This belief in demonical possession as a cause of disease figures in the New Testament. The story of

plague maiden (D' Irsay, 1925) spreading plague was in vogue even as late as 1878. Numerous devices were employed to propitiate the demons or to transfer the evil by sorcerers, magicians and by witchcraft in Europe and Arabian countries till as late as the eighteenth century. Thomson (1666) described 'toad' medicine for cure and prevention of plague following the London plague in 1665. According to Paracelsus (1490-1541) a specific medicine such as 'toad' medicine operated by exerting a kind of occult sympathetic action on the essential nature of such a lesion as a bubo. Thus a school of mystic practitioners of the occult art of healing prevailed and is even present till today. From the age of Babylon to the present day amulets and talisman believed to be impregnated with occult power have been much used for prophylaxis against plague and other epidemics. At the lowest level of human intellect, a belief in the efficiency of magic and magico-religious charms, is still the universal faith of all mankind, the truly catholic creed (Rivers, 1924). In any case, it has to be admitted that modern science has evolved from these magical and occult ideas and practices and doubtless many of our current concepts have arisen out of logical and natural association of the system of ideas of primitive man combined with a dim intuition of the unity of cosmos.

Wrath of God as a Cause of Plague

The record of the first of all indubitable plague epidemics among the Philistines and Israelese shows how when two different nations were affected together, one sought deliverance by magic and the other by prayer and sacrifice to God. It is in these epidemics that the relationship of plague with bubo in human subjects and in mice and rats and the epidemics with 'emerod' (bubo ?) swelling in the vicinity of secret part (groin) was suggested. While the Philistines deified natural forces and appealed to demons to deliver them from the pestilences, the Israelese believed that pestilence was due to the righteous anger of one Supreme offended Deity-the true God scourging his chosen people for their sins and they sought in repentence prayer and sacrifice for deliverance.

In the Book of Psalms it is written 'God judgeth the righteous and God is angry with the wicked everyday. If he turn not, he will whet his sword; he has bent his bow and made it ready'. In the Art Galleries of Europe there are many paintings illustrating the Wrath of God following the Roman plague of 589 A.D. Mahomedans, too, were sure that pestilence was a punishment for sin and could not be averted. Similarly, among the oriental people the belief is still active that epidemics of all kinds represent the vengeance of the angry Gods on disobedient people and they attempt to appease Gods when such epidemics appear (as in case of smallpox, cholera and the like).

During the epidemic plague in London, it became increasingly difficult to reconcile the natural with the supernatural view of plague. Before the end of the 16th century the British Civil Authorities were often embarrassed by the activities of the clergy. In other words, with the rise of knowledge about

the spread of plague there was a conflict between the claims of religion and the budding sanitary science. In fact, a London Clergyman named Henoch Clapham was sent to prison in 1603 A.D. for publishing a pamphlet which affirmed that plague was punishment for a sin and that death was a consequence of lack of faith. However, in prison he explained that the causes of plague epidemic were both natural and supernatural. The plague due to natural corruption was infectious but one due to angel's stroke was not. The belief held the ground throughout the Great London plague and the Clergy continued to preach sermons calling large congregations to repentance and to join in prayer imploring God to stop plague.

Plague Poison Spread by Human Agency

The 'Black Death' was unique for its variety and extent of the pathological effects it produced on the minds of men, and an unusual collective psychology emerged in addition to the unparalleled disturbance in men's minds. On the one hand, some groups spread the cult of repentance for the sin *e.g.*, by the Brethren of Cross and on the other, collective mental aberrations assumed far more sinister shapes, rousing, as they did, the basest of human instincts and sweeping away every vestige of reason, justice and mercy. The worst example was the persecution of the Jews as the originators of the epidemic and atrocities committed on the alleged purveyers of plague poison. In Southern France (1348 AD) the rumours crossed the Pyrenees that Jews were causing plague by poisoning the wells. It spread over Hungary, Italy, Poland, England and Germany. All classes of the community bound themselves by oath to extirpate the Jews by fire and sword. Thousands were burnt alive. Wealth fell into prosecutors' hands and confessions were extracted under excruciating torture. It was through the intervention of Pope Clement VI and Duke Albert of Austria that the Jews as a race were saved from complete annihilation.

On a similar plea the English were suspected as plague makers in France and the French in Italy. Even grave diggers often fell under the suspicion of ushering in plague. The general view was that plague poisoning was a relic of the magical black art of older times. "Plague anointers" were supposed to have played a part in initiating the great epidemic of 1576 in Milan by streaking the deadly compound on street doors and walls. This led to the celebrated trial of Piazza in which the plague overseer, and Mora, the barber were charged of spreading plague. After extracting confession by repeated torture, they were killed with hideous cruelty (Sticker, 1908). The trouble began with the spread of rumours that the French and the English authorities were despatching conspirators to poison the people (Fletcher, 1898). This kind of mass hallucination about poisoning in plague season was, however, not seen in Bombay in 1896 when plague broke out there, but stray accusations against the British officials by malicious agitators seem to have been noted.

Miasma and Contagion

Over four centuries before the birth of Christ dawned the idea of the natural causation of disease in the island of Cos (Greece) in the teachings of Hippocrates, known as the Father of Modern Medicine. He was the first to insist that the true function of the physician is to assist the *vis medicatrix natural*, rather than to practice magic or to invoke anthropomorphic Gods. However, the priest-physicians still conducted worship in the temple of Aesculapius according to the ancient rites, but the origin of epidemics began to be related to terrestrial changes suitable for study on objective lines and thus medicine gradually ceased to be all mystery. Eventually three main theories developed to account for the origin of epidemics. They were attributed to *miasma*, *contagion* or *astral influence*, each of these being still considered to have some supernatural aspects.

Pollution of air by noxious vapours was supposed to be the cause of pestilential diseases. Such ideas prevailed for 2000 years on the authority of Hippocrates and Galen. At the time of Black Death, the theory of miasma and contagion was reviewed and for a prolonged period the battle between the miasmatists and contagionists continued until early 19th century when epidemics of plague became increasingly irregular and virtually confined to certain plague spots in the Middle East. Hence the conception of a widespread atmospheric poisonous influence, due to an astral or general telluric cause, became inadequate to explain the facts and a series of 'localist' hypotheses were developed. Thus plague was said to be due to noxious emanations from soil polluted by the decay of human corpses, and its germs were spontaneously generated under grossly insanitary conditions, foreshadowing the theory of spontaneous generation sometime later.

Astrology and Plague

The 'Black Death' smote Europe at a time when the minds of physicians were saturated with the astrological lore. This continued to have a dominating influence on the British as well as the contemporary continental medicine till the beginning of the 17th century. Throughout the 14th century, however, the leaders of the profession firmly believed in *astral influence* as a prior cause of plague, but thought that *contagion* and *miasma* also played a more immediate part and there was always the *wrath of God* to be reverently considered. In other words, the people were then open to entertain any and every hypothesis concerning the origin of the great calamity and often in the plague tractates of the period a variety of causes were accepted as being simultaneously or successively in operation. In his *Grande Chirurgie*, the first systematic text book of surgery, Guy de Chauliac gave a brief but very valuable account of the 'Black Death' and attributed the original cause to the conjunction of Saturn, Jupiter and Mars in the sign of Aquarius on March 24, 1345 (Vinario gives the data as March 28). Others believed that the State of Heavens exerted only a remote predisposing effect on plague and was the immediate cause of pollution of air (Singer, 1916; Sudoff, 1348).

The most elaborate exposition of the astro-miasmatic point of view is contained in a report on 'Black Death' issued in October 1348 by the College of Physicians of Paris prepared at the command of the King Philip VI of France, commonly known as the *Compendium de epidemica*. The University of Paris at that time was a stronghold of both astrological medicine and orthodox theology (Sudoff, 1348).

The essence of the theory of origin of 'Black Death' seems to be that the state of heavenly bodies (triple planetary conjunction) led to the pernicious pollution of the air through vapours drawn from the Southern seas and carried as a fog with great dampness and heat and this lasted as long as the sun remained in the sign of Leo. This pronouncement of the Paris Faculty influenced many writers of plague for the next hundred years. A detailed exposition of the planetary effects on plague is to be found in a tract issued by the quack astrologer, Gadbury (1605). He ridiculed miasma as a cause of plague and declared heavens as the giver of 'efficient and essential plague'.

Flaming comets and showers of meteors were commonly considered as the precursors of plague. Three comets are said to have preceded the advent of plague of Justinian, including the blazing Halley comet which illuminated the sky for 20 nights in A.D. 531. Plague was associated with comets in Scorpio and Pisces.

In 1664, Engelhard predicted plague in autumn on the appearance of a comet in the Russian sky plus a conjunction of Saturn and Mars. As a matter of prevention, the Czar banned the English ships to arrive at the Russian ports. Russia escaped infection, but London had it in 1665 (Sticker, 1908).

Whatever views the contemporary medical observers of 'Black Death' expressed regarding the ultimate cause or causes of the great epidemic, all agreed on the deadly danger of contagion. Thus, in spite of their astrological bias, the Medical Faculty of Paris warned the healthy to avoid poisoned air exhaled by the sick and to keep away from them, and so did Gentile da Folings (Sudoff 1348), Ibn Khatimah and Ibn-al-Khatib who believed also on the will of Allah. It is thus highly probable that the high infectivity of plague became well established in consequence of the tragic cause of the 'Black Death' when man to man infection (pneumonic type) was obvious.

Pneumonic plague seems to have been a striking feature of the early phases of the plague epidemic in Central India in 1812 and 'Pali' (Rajasthan) Plague in 1835. Moreover, when the last pandemic reached India in 1895 primary pneumonic plague was already fully recognized. As described by Childe (1897) small outbreaks of this type were not infrequent in Punjab, Ceylon, Java, Madagascar and Burma though the proportion of death due to pneumonic plague was less than 1.0 per cent of the total deaths due to plague. It was not until the great Manchurian pneumonic plague epidemic broke out in 1910-11 causing 60,000 deaths that this type of disease was intensively studied. However, when plague broke out in Bombay, people still wore lockets containing camphor, eucalyptus and other aromatic substances. Tobacco smoke came into use in Stuart's time as a plague prophylactic, though it was more

usual to chew the leaf. Diemerbroeck in his great treatise on plague states that the tobacconists of Nymwegen were free from plague during the epidemic in that town in 1635, and therefore recommended tobacco smoking as a safeguard against infection. These remedies were originally based on Miasmatic theory.

After mankind emerged from the Dark Ages, the conception of contagion began to take shape and the impression lasted long after the disease in its bubonic form had ceased to be contagious in the ordinary sense. Flight from the place of epidemic as a protective measure was recognized early. Thus cardinal Wolsey recommended escape into clean air as the sole effective safeguard against plague infection. In England, after the disease became endemic in the big towns, it became the custom for the Court, the nobility and the wealthier merchants to kept up and go off into the country whenever plague showed a tendency to effervesce into unusual activity in summer time. The cavalcade returned with the onset of winter.

By the 15th century three kinds of contagion were recognized, namely, by contact, by formites and at a distance. No one including Galen before Fracastorius (1546) seems to have given serious thought to the implications of the idea of contagion which was inseparably linked in men's minds with both magic and miasma. Contagious persons were foci of miasmatic influence, as opposed to the radically different and moler germ theory of disease. According to the latter, each infected individual becomes a new and often mobile factory of self-generating living organisms, being capable of initiating a new epidemic under conditions favourable for the transmission of the germ. Although Fracastorius' mind was saturated with the ancient humoral pathology, it was through his extensive experience of outbreaks of plague, typhus and syphilis that he became sceptical about Galen's epidemiology, especially as it failed to explain why healthy people with healthy humours were infected *en masse* simply by contact with the infected persons or even with their clothes, although there was nothing favourable in the atmosphere or in the bodily condition. Fracastorius therefore postulated invasion of the tissue of the body by invisible perticles precisely similar in nature of infected and the infecting persons. But this idea was vitiated by the thought that initially these infective particles might be generated by putrefaction in some unhealthy person or might originate spontaneously in foul air or stagnant water, *i.e.* the disease might arise *de novo* or by spontaneous generation. The ideas of Fracastorius were thus not entirely free from miasma or influence of heavenly bodies.

Miasma Versus Contagion

Greenwood (1921) remarked in an address on Galen as an epidemiologist-'It is certain that a fear of contagion in all forms of evil is immensely old'. Isolation by religious taboo was probably practised from time immemorial among the Hindus, Parsees, Chinese and Egyptians. Manu's Laws of Health in India are several thousand years old. It is also clear from

Thucydides' account of the plague of Athens in 430 B.C. that it was thought to be exceedingly infectious. Evagrius believed Justinian plague to be contagious but there is no referencc to contagion in the whole of Hippocratic collection.

According to Galen, contact with plague-stricken individuals was as dangerous as it was with patients of ophthalmia, itch or pthisis or with any one who expires bad air. This is an early version of what may be termed the miasmatico-contagious point of view favoured until the end of the nineteenth century by many writers on plague. During the Middle Ages it was supposed that if the humor of the patients became sufficiently replete with pollution they could concentrate the miasmatic poison in the air around them, exhale it in their breath or emit it from their bodies, so that not only actual contact but also near approach was risky.

During the fourteenth and fifteenth centuries, the prevailing conception of the nature of contagion was one which could readily be reconciled with the classical miasmatic point of view. It was mainly a question of degree of emphasis to be laid upon the various factors which were believed to be in simultaneous operation including divine power, astral influence, miasma and contagion. For instance, while Khatimah rejected the idea of contagion on religious grounds, Ibn-al-Khatib, the contemporary statesman-physician did not hesitate to assert that the disease was chiefly transmitted by infection although he did not exclude miasmatic influence as a factor in the spread of disease. He also admitted purely local or special pollution of the air in the vicinity of the plague-stricken houses. He also noted the advantages gained by cities which forbade the entry of travellers from the plague-stricken areas.

According to the learned medical historian Haeser (1882), the war between the contagionists and anti-contagionists was most fierce during the 16th century. At this time, although the medical profession was sharply divided into two groups, most physicians seem to have been willing to compromise and accept both the hypotheses, *i.e.*, plague originated spontaneously in an unhealthy atmosphere and, others thought that it spread by contagion *i.e.*, a miasmatico-contagion view (Creighton, 1891).

Gradually, the idea about the mode of spread became clearer, particularly following plague in Venice in 1576. Massaria showed that the infection was imported from the city of Padua and its spread could be traced from place to place. According to the miasmatic view it should have broken out simultaneously through the area affected. Moreover, he showed that efficient isolation of patients was effective in controlling the spread of the disease.

The experience of London epidemic of 1665 helped to clarify further the mode of transmission although difference of opinion was simultaneously existent. Hodges (1672) believed that plague was contagious and transmissible by importation of infected grains (*e.g.*, cotton and silk from Levant to Westminster in London via Holland). Beghurst (1666) put forward the original version of the miasmatic theory foreshadowing the 'localist' theories of

the early nineteenth century which asserted that the disease was latent in London and descended like a rain on a number of separate localities. Cases of plague had occurred for three or four years previously and the Bills of Mortality had recorded burial of persons alleged to have died from plague every year since 1665. Before that there were six such deaths in 1663 and nine in 1664 followed by 100 deaths per annum till 1669 (end of Civil War).

It may, however, be questioned now whether the great plague in London was due to recrudescence of a long-standing smouldering endemic disease in the modern sense or to the introduction of new or more virulent or infective strain of the organism imported from overseas, as in 1663 plague was raging in Levant and Danbary coast which spread to the Coast of Greece, Italy and France and reached Amsterdam in 1664. Ships from the Dutch port were quarantined in the Thames in June 1664, but Yarmouth which had a close commercial intercourse with Holland, was infected in 1664 before London. Although it is difficult to support any of the above two possibilities with surety, the recrudescence was no doubt a possibility from the experience the author had in Calcutta epidemic of 1948-1952. He made various experimental studies which supported the theory of recrudescence of the last plague epidemic in Calcutta (Chapter VI). The same might have happened in London as seen from the available records. It is of course now known from our Indian experience that both factors play a part, recrudescence of infection being more common in the larger towns and reimportation in the smaller villages.

The great epidemic of Marseille (1720-21) revived the opposition to contagionist doctrine. The situation particularly arose because the majority of the human plague cases were of bubonic type and man to man transmission did not take place. It was, however, clear that the infection was imported from Levantine coast in the ship (Grand Saint Antoine). A severe epidemic broke out at a Syrian and other ports shortly after the ship left with *portents nette*. The infection was clearly brought in the first instance, to Marseille from Overseas on shipboard.

Miasmatic notions about volume of foreign pestilential air being wafted from the holds of the ship to quays was not accepted. There could be no reasonable doubt that it was the seeds of the disease to the porters who first handled the goods and then to the merchandise in Toulon (D' Anterechaus, 1725). In the light of the present knowledge of epidemiology it is reasonable to infer that a severe rat epizootic occurred in the hold of the ship (Grand Saint Antoine) and that many plague infected fleas released from the bodies of the dead rats took refuge in the interstices of the various types of goods or in the covering of the bales. These fleas were carried ashore to slake their thirst on man and later on local rats to initiate an epizootic among them later spreading the infection to the human beings.

In the opinion of Pestalezzi, (17-21) an outstanding contagionist discussing Marseille epidemic, 'the infection might have spread *per contactum, per*

fomiten or *ad distans* by the exhalation of the volatile principle'. Mollier (1885) one of the plague commissioners of Lyons, also favoured *contagium vivum* as the infection agent. Mead (1721) of England propounded the theory that contagion was propagated in threefold manner—by air, by diseased persons and by goods transported from infected places, and entertained the view that plague in England always came from foreign ports through infected goods (*e.g.*, cotton from Turkey). Accordingly he advocated a system of quarantine on Venetian lines in order to prevent the importation of infection from abroad. This view was challenged by Pye (1721) on the issue how goods could be infected unless these were in the end-chamber of the sick. This point could not be clarified till the theory of rat plague and infected fleas was known.

Eggerdes (1721) waged successful campaign against plague in Silesia on strict contagionist lines. He traced the spread of plague from Hungary to Turkey and then to Austria, Germany and Poland. According to him, the infection was carried in fomites and the poison was capable of multiplication to infinity. A full discussion of the 18th century controversy concerning the infectivity of plague and especially of the part played by merchandise in transporting the germs was expounded by Russell (1791).

Plague in Malta and Ionian Islands in June 1819 revived the theory of contagion by Brooke-Faulkner (1820). This was criticised by White (1846). Maclean (1817), a physician of East India Company having some experience of plague in a Greek Hospital, held extreme anti-contagionist view maintaining that quarantine lazarettoes and all attempts of police control of the disease were useless and that all epidemic declined in the natural course of events as in Gibraltar. In the Egyptian outbreak in 1854-55, the leading opponent of contagionist theory was Clot-Bey (1841) a pioneer of Western Medicine in Egypt and published a book in 1840 with detailed description of orthodox miasmatic doctrine of disease (that atmosphere becomes charged with plague producing power) with synopsis of views of other authorities in this controversy. This was severely countered by Bulard (1839) who had experience of plague in Constantinople and Smyrna as well as Egypt. He was one of the first to suggest that quarantine period of plague suspected goods could be greatly shortened by heating them to a moderate degree over a stove to disinfect the plague poison.

The champion of contagion at that time was, however, Grassi, Medical Officer in-Charge of Alexandria lazaretto, as also the German Doctor Fischer who postulated that the sporadic form was free from contagion but during epidemic an infectious principle developed.

Human Experiment

Interestingly, to establish each other's point and to settle the dispute once for all, several bold inoculation experiments with plague material on human subjects were carried out in Cairo. Since they were carried out in an epidemic atmosphere Clot-Bey had to admit the results as inconclusive.

In one experiment a condemned criminal slept in a patient's bed wearing his sweat-saturated under clothes. This person developed plague and died. Another criminal under the same experiment developed inguinal bubo but recovered. Fresh blood from the cephalic vein of a second day patient was inoculated into the arm of another criminal, an immature bubo developed which subsided without suppuration. The second criminal was inoculated in groin and axilla without ill effect. Another friend with repeated inoculation with bubo pus gave negative result. Then Bulard wore shirts of a plague patient for 48 hours. Clot-Bey inoculated himself with blood of a patient, six fairly deep scratches being made into the skin of groin and forearm. No symptoms developed in either case, though the patients supplying the material were severely ill at the time and died. Experiments on animals were also conducted, dogs being heavily inoculated with a variety of plague material without result. Several points are to be considered for interpreting the results. Dogs are generally resistant to artificial infection with plague. Bubo pus often contains few or no organism and such organisms are not generally present in human bubo before the terminal phase of the disease. Besides case mortality in this particular epidemic was low, the epidemic being clinically mild. However, Clot-Bey, like all other predecessors of miasmatic theory, believed that flight from the scene of the epidemic was the only and sure way of salvation and that none save those with essential duties should remain. These people should avoid fatigue and excesses of all kinds and pay attention to the rules of hygiene and try to keep up their morale. Thus based on the above experiments and other observations, we now know that there was truth as well as errors on both sides. The infection in bubonic plague is not contagious but its germs are often transported in certain kinds of vermin-infected goods. On the other hand, residence in plague houses is very risky not because of the contact with the sick inmates, but because of the proximity of plague infected insects with an appetite for human blood.

It was true that the disease could flourish under certain favourable climatic conditions. From the point of view of history of epidemiology, this Egyptian experience is interesting because attempts were made to solve the issue by experiments even on the persons of the investigators. However, the controversy between the contagionists and miasmatists continued and it was thought necessary to decide whether quaranite should continue in full force in the Mediterranean ports. The question was discussed in a meeting at Paris in 1844 and by a Commission of the Royal Academy of Medicine attended by Clot Bey and other physicians.

Concept of Specific Maladies and Etiology of Disease

Not many years ago, during the 18th and early 19th century, a belief was still extant that the various kinds of epidemics were mere phases in a general infective process which could be transmuted and the idea that each infection was due to specific germ was ridiculed in many quarters. In fact,

the word "pestilence" covered almost every kind of acute epidemic. It was not until the first half of the 19th century that febrile infections were clearly distinguished one from the another by their clinical signs and morbid anatomy. Sydenham (1676) was one of the first to advocate the rigid classification of diseases according to the species based upon his unprejudiced clinical observations at bed-side, yet the theory of mutable epidemic constitution was scarcely compatible with his description of specific epidemic diseases. Bulloch (1938) who contributed much to our knowledge of epidemic fevers wrote that there existed a strong analogy, if not identity, between typhus and plague, the poisons being generated from similar causes differing only in intensity. The confusion between typhus and plague is an old one. Creighton (1894) in as late as 1890 wrote that when ordinary typhus generated by unhealthy air passed into a soil poisoned by aggravation of conditions it became at the same time bubonic fever or approximately plague proper. At that time there was a group of authorities who believed that all epidemic diseases could transform themselves into plague at the acme of the epidemic; Hirsch (1881) pointed out that when plague, typhus and malarial fever were prevalent together, as at the time of Balkan War, at the end of the 18th and the beginning of the 19th centuries it was natural to assume some nosological or etiological connection between these three diseases. Haeser (1882) noted that Orracus of Moscow believed in the gradual evolution of plague from typhus and that smallpox and quartans might appear in the declining phase of the epidemic.

The differential diagnosis of specific communicable diseases progressed on three lines namely, first, on nosology based on symptoms and signs observed at bed-side (by the eighteenth century physician); second, rise of knowledge on pathological anatomy (at the beginning of the nineteenth century); and third, classification of diseases according to the distinctive appearances seen in the post-mortem room. According to Bulloch (1938), Bretoneau (1922) may be regarded as the real founder of the doctrine of the specificity of disease as now understood. His studies led him to the conclusion that the etiological agent of each disease is specific. Infection must be a contagious principle or reproductive agent, though he did not compare his *causes materialles extrinsigues* to a vegetable or animal micro-organism. Clinically, plague is far from being a homogeneous disease, including, as it does, such diverse types of clinical manifestations as bubonic pneumonic, septicaemic, cutaneous, variolous and tonsillar. The demonstration of an infective agent common to all these varieties of clinical conditions had to await the development of modern bacteriological technique. The disease was so much dreaded particularly following the European episode of 'Black Death' that almost invariably any announcement of the advent of the dreaded scourge excited and even now excites a hostile popular reaction and was and is still not welcome by either the health or the commercial authorities. The doctor courageous enough to diagnose the first case in commonly denounced. History abounds with such incidents. The initial diagnosis of plague

in Bombay in the autumn of 1896 was very reluctantly accepted. The same position repeated itself in case of the present author when he declared plague in Calcutta in 1948. It was indeed a great uphill task for him to convince the corporation and governmental health authorities and even high ranking medical men to accept the diagnosis of the initial cases. The epidemic however persisted till 1952, in spite of the control measures taken.

Origin of the Germ Theory of Disease

It was Fracastorius (1546) in the sixteenth century who could throw much light on the mechanism of the contagious process, but the true nature of the infective agent remained obscure. For ages contagion had been treated as either animist or physical phenomenon. It was not until the 16th century that the idea of its being associated with living organism was seriously entertained and not until the nineteenth century the germ theory took a concrete shape or became generally accepted, although the crude concept of a living infective organism was immensely old *e.g.* the doctrine of *contagium vivum* from 1500 to 1750 A.D. With the development of simple magnifying lens and at the initial stage of development of the compound microscope by Leuwenhoeck (1632-1723) that actual search into the nature of hypothetical minute *contagium vivum* became possible leading to the final success for the first time during the middle of the nineteenth century. The first explicit claim to observation of a living micro-organism causing plague was made in 1658 by the Jesuist Priest, Athanasius Kircher in "*scrutinium physico-medicum contagiosae luis quae dicitur Pestis*". He possessed a compound microscope with coarse and fine adjustment but the lenses did not give enough definition to enable the plague bacillus to be recognised. Kircher was however considered as the father of bacteriology and a pioneer of the germ theory of disease.

Soon a counter current was set up with the discovery of miscellaneous microbes both in the healthy and the diseased tissues alike and a tendency developed to look upon them as results rather than causes of disease. The cogent argument in favour of the germ theory of infective diseases put forward by van Plenciz (1705–1786) in his *Opera Medica Physic* published in 1762 failed to cut any ground, as in the existing state of knowledge of that time it was almost impossible to refute such criticism, with the result that during the end of the 18th and beginning of the 19th century, there was a temporary eclipse of the germ theory of the disease. The belief of spontaneous generation (Redi, 1688; Needham, 1745) was an obstacle to the acceptance of the germ theory. On the other hand, Mollier (1885), Marten (1901) and Bradley (1921) of the 18th century and a series of 19th century workers including Pasteur refuted the ancient belief in abiogenesis.

Micro-organisms and Epidemic Diseases

It was not until the 4th decade of nineteenth century that interest in the germ theory was revived again and in the seventies, Pasteur, followed by

Koch a few years later, succeeded in isolating and fully describing pathogenic bacteria and demonstrating that these could cause disease in man and animals.

During the first half of the nineteenth century, Henle (1840) was the leading champion of the germ theory of contagious diseases but in the absence of identification of the germ he alloted an important role to the miasmatic factor in most epidemic diseases, including plague, which he thought might appear miasmatically and then spread by contagion. Accoring to him some diseases were purely miasmatic *e.g.*, ague (fever with shivering), some were purely contagious *e.g.*, scabies, syphilis, hydrophobia, *etc.* and some others were miasmatic-contagion *e.g.*, plague, typhus, *etc.* Agostino Bassi followed by Latour (1836) discovered the first fungus causing the silk-worm disease-Muscardine. Schwann (1837) and Kiltzing (1837) established that fermentation by means of yeast was due to the activities of a living organism and not purely a chemical process as it was formerly thought (Bulloch, 1938). About this time Schonein discovered fungus of favus-a skin disease of scalp and this lent further concerete support to the theoretical reasoning of Plenciz and Henle. Henle advanced three postulates that must be fulfilled before any micro-organism could be accepted as the cause of a particular disease *viz.*, (i) the germ must be regularly found in the contagious material from the human body; (ii) it must be capable of being isolated from the material; and (iii) when isolated it must be capable of reproducing the disease in another human being. It was more than thirty years later that one of his pupils Robert Koch, was able to fulfil the conditions and furnish definite proof that Henle's theory was correct. Henle's difficulties were the theory of spontaneous generation and lack of technique for isolation and identification of the organism. Louis Pasteur (1822-1895) and his colleagues inflicted the final blow to the doctrine of spontaneous generation of living organisms (abiogenesis) by the crucial discovery that some bacteria generated highly resistant spores which could be destroyed by heat at 100°C and the material if kept in tightly corked flask in which it is boiled, remained sterile for as long as the supernatant air was kept free from germs.

The next difficulty of isolating the organisms in pure culture was overcome by the discovery of solid culture media contained in glass tubes or covered plates with agar—agar as solidifying media of Hesse. Simultaneous improvement of the powerful lens in the microscope also helped in studying the organisms by staining process. Around eighth decade of 19th century, the progress was rapid; the germs of cholera, tuberculosis, diphtheria, typhoid and many other epidemic diseases were isolated and described largely under the leadership of Koch, while Pasteur pioneered the production of immunity against microbial infection by inoculation with vaccine. Thus the way was finally opened to the almost simultaneous discovery of the plague bacillus in Hongkong by the pupils of Koch and Pasteur namely Kitasato and Yersin.

Even so, the germ theory of infective diseases was slowly accepted by the

great majority of the conservative medical profession. Even at the beginning of the present century, some British epidemiologists believed that the so-called specific organisms isolated in bacteriological laboratories were mere consequences rather than causes of the epidemic disease. Payne (1886) writing on plague in 1885, when Koch had already established the new science of Bacteriology, wrote of an organised poison (or bacteria) growing in insanitary conditions outside the human body; but all the emphasis was on a chemical poison rather than a living germ. Creighton (1894) thought of plague as due to poison emanating from putrescent corpses. Such opposition was also offered by Bastian a defender of abiogenesis, in 1875.

The gradual change in the informed opinion was clearly discernible beginning from the second half of the nineteenth century following the long series of annual report by Simon (1887) the greatest public health official ever produced by Great Britain. If his series of reports is studied, it will be seen that in the course of thirty years beginning from 1848 Simon progerssed steadily, step by step, from the conception of miasma emanating from putrefying filth of every description to a specific chemical poison in both air and water derived from the pollution of special kind, to a dead specific enzyme, and finally, to the modern view that specific infections were due to the effects of invasiveness of the tissues of the body by specific microorganism. The contamination of water through discharge from bowels was thought to be the cause of epidemic and with him marched majority of the contemporaries, though with slow pace. His ideas however, considerably influenced John Snow's (1936) classical work on cholera and Budd's work (1873) on typhoid. However, Koch and Henle's criteria for genuine specific microorganisms were not enough for Homer (1928) who followed Hottinger (1903) in believing not only that an animal inoculated with pure culture of the germ would develop the disease, but also that such sick animals should prove infectious to others of their kind. That is, the artificially produced disease must fulfil the requirements of epidemiology. In respect of plague this requirement was fulfilled by producing an artificial epizootic among groups of healthy rodents which closely resembled natural plague among the same species of animal with the same symptoms and post-mortem signs. This was further confirmed by an actual outbreak of pneumonic plague in a pathological institute in Vienna in 1898, initiated by a laboratory assistant who had attended animals artificially inoculated with *Yersinia pestis*, the plague germ, and later spread among doctors and nurses.

New Versus Old Concepts of Epidemiology—Causal Connection

At this stage controversy had arisen between the old and new schools of epidemiology regarding the significance of the term 'cause'. It is difficult, if not impossible, to envisage a genuine causal connection between a series of distinct happenings so that anyone is a necessary consequence of that preceding it. Science can only describe regularities in the sequence of events and make useful predictions accordingly. There must be an all-embracing

process in which event—past, present and future, depends on every other. Causation implies the operation of a vector—a directive activity. The idea of direction is really implied in that ambiguous expression 'force', even though we are not at all sure what the term really means. Again, the word 'causes' is itself ambiguous. In one instance say, A is necessary though perhaps not sufficient condition of B; or a sufficient though perhaps not necessary condition of B. In another situation a set of conditions may be severally necessary and jointly sufficient to produce B.

Specific micro-organisms are said to cause epidemic diseases, but they may equally well be described as causes of immunity to the same disease and therefore act as cause of its decline. For instance, in the epidemiology of airborne diseases, such as diphtheria, both infection and immunity may be produced by aerial exposure to diphtheria bacillus. Again, the result actually depends on the quantum and frequency of this exposure and the virulence of the organism. For instance, repeated exposure to small number of microbes for short periods of time may result in building up immunity to the disease, whereas a massive and prolonged exposure of the tissues of the respiratory passages of susceptible persons may result in severe attack of diphtheria. Similarly there are strains of plague bacillus so virulent that injection of a single organism beneath the skin may suffice to kill a guineapig; on the other hand, there are strains so lacking in virulence that an entire culture containing millions of bacilli may be inoculated into guineapig or human being without causing any illness. In other words, some organisms carry a pathogenic factor, others do not. For instance, 'V' antigen of typhoid organism may be termed the infection factor while in the case of plague virulence seems to be linked with the envelope substances (Meyer, 1950a). In Java and Madagascar a large contingent of population was protected against plague by inoculating them with vaccines made of live (attenuated) plague bacilli. Thus the rise and fall of the average epidemic disease among a community of men or animals reflect a continuous interaction between host and parasite, between the quantum of infection and the quantum of immunity. Not only does the host react to the invasion of the parasite by falling sick or becoming immune, but the parasite may itself react by changes in its infectivity and virulence. These are often associated with only one phase of the development of a specific micro-organism and the two characters may vary independently of one another. Striking variations in the bacterial structure (protoplasm) may take place which are commonly termed as 'dissociation' and which even permit the organisms to remain within the host to act as 'carriers'.

There is no doubt that pioneers of bacteriology maintained strict specificity and invariability of the germs they discovered and the diseases caused by those germs, and somewhat crude concept prevailed for the interaction between host and parasite. Hadley (1928) has described the successive stages in the evolution of modern view, according to which microbes of a given species vary considerably though in a characteristic manner in definite direc-

tions. The bacterial cell is now known to be built up of variable amounts of separate components (antigens), each containing a distinct immune response (antibody) when injected into a susceptible animal. Thus the pathogenic powers of bacteria are now known to be associated with particular types and phases in the life history of a mutating organism. The technique of antigenic analysis of pathogenic bacteria has become increasingly elaborate in recent years *e.g.*, classification of bacteria on bacteriophage sensitivity. However, one remarkable feature of plague by contrast with cholera, typhoid and dysentery is the relative constancy of the strains isolated from human cases irrespective of clinical manifestation of the disease. No distinction could be made between the organism isolated from pneumonic cases and those isolated from bubonic or septicaemic cases, although different categories of strains have been obtained from artificial culture media as well as from the rodent carriers. But no healthy carrier has so far been discovered in human beings. We must therefore agree with Homer (1928) and Crookshank (1922) that it is still difficult to account for all the phenomena of epidemics in terms of reciprocal interaction of a community of hosts with their parasites. Similarly till the middle of the present century an adequate explanation was still lacking on the appearance and disappearance at long intervals of time of the grand pandemics from the stage of history. Even as late as 1917 Klebs and in 1919 Greenwood emphasised on the intensive study of the many factors causing epidemics and pandemics.

The author's own work (referred to later in this book) has served to further clarify the position with regard to the identification of different types of plague organisms and their role in the epidemicity and endemicity of the disease and their antigenic and immunogenic characters.

This chapter thus summarises the phases I and II of the evolution of knowledge on plague, chronologically starting during the primitive period, steeped in superstitious beliefs, concepts of miasma versus contagion, progression to the natural causes of diseases and culminating in the germ theory of epidemic diseases and finally the unfolding of the cause and mode of transmission of infection by careful observations, experimentation and reasoning. Phase I persisted till the 4th century B.C., when Hippocrates propounded the theory of the natural causes of diseases.

Phase II was influenced greatly by the naturalistic theory of miasma based on first hand observations of the effects of climate, season and locality on the incidence of outbreaks of disease as well as on the physical and mental characters of human beings. Even so, the great mortality from "black death" led to collective mental observation, nursing even the meanest of human reaction and sweeping away every vestige of reason, justice and mercy. As a result the Jews were persecuted as the originators of epidemics and purveyors of plague poison. The English were alleged to be plague makers in France and the French in Italy. The general view was that the plague poison was the relic of magical black art of the earlier times. The ideas were mixed with miasma, contagion and astrology which, besides various types of super-

stitions, held the ground for many centuries. However, the contagionists were beginning to think about the communicability of disease through their conception of *contagium vivum* and those propounding the 'localist' theory were also partially correct, as bubonic plague did turn out to be a disease of locality.

Local filth and insanitation was, however, a means of generating plague only in so far as it also generated rats. Similarly if soil was a reservoir of infection it was only because it sheltered rats and fleas. The miasmatics were on the other hand, wrong in believing that the air was poisonous in plague time, while Hippocrates was right in attaching great importance to weather as a factor in the spread of epidemic disease.

The warmth and moisture associated with the so-called pestilential constitution promoted the multiplication of the carrier insects. In other words, the vague ideas or theories that crept into the minds of the people gradually became clear and the true nature of the cause and mode of transmission of infection unfolded itself by careful observation, experiment and reasoning. Thus ended the period of the second phase of evolution of knowledge on plague.

CHAPTER IV

Phase III of Evolution of Knowledge on Plague—Beginning of Modern Research (Source and Transmission of Plague)

Phase III was the beginning of modern research which finally exposed the natural modus in the ecology of the disease with most of its observed nature clarified and known. This phase extended from the period following the theory of contagion and role of rate to the end of 19th century (1894) when the causative germ of plague, *Y. pestis*, was discovered by Yersin and Kitasato followed by introduction of anti-plague vaccine by Haffkine as a prophylactic measure against the disease.

The Role of Rat in Plague

The earliest record of probable relationship of rats to plague, as mentioned earlier, is found in the Indian scriptures like the *Bhagvata Purana* (1500-600 B.C.) which refers to the death caused by an epidemic disease preceded by an enzootic among rats. The next reference of rodents (mice) to plague was in connection with the outbreak of the disease among the Philistines in 1320 B.C. (Wu Lien-Teh, 1936). The Bible mentions 'mice' but the Hebrew word 'akbar' might mean rat. In all probability the mice in the Philistine towns and ships were really rats (*R. rattus alexandrianus*). In fact many votive offerings in the form of mice have been found in Alexandria, Palestine and elsewhere. However, references to any association of rats with plague in the classical, mediaeval and even the seventeenth century literature of Europe was practically absent unlike that of Asia where according to the ancient tradition mortality among rats was a harbinger of death of man. Cuvier (1769-1832), the great Zoologist of the 19th century suggested that there was no rat in Europe earlier than the sixth century A.D. Accordingly, the great plague of the sixth century could not have originated from the rats. Similar question may also be raised for the 'Black Death' of the fourteenth century. The latter had, however, a very high proportion of pneumonic deaths and septicaemic cases indicating quick man to man transmission. Bubonic cases were also recorded though generally highly acute and fatal. In the opinion of many writers, rats were absent even up to 12th century

and were naturally absent during the Justinian plague (542 A.D.). But it is generally admitted that mice were already abundant in the West at that period (Shrewsbury, 1949; Girard, 1950a) though they were supposed to be only secondarily involved (Tricot-Royer, 1950). However, this opinion was not shared by MacArthur (1952) who adduced evidence pointing to the early presence of rats in Europe. Judging from the records of all the three pandemics it seems improbable that rats were not involved. On account of this uncertainty about the mode of propagation of plague in the 14th century, Martin (1913) and Jorge (1913) postulated that human flea (*Pulex irritans*) which had been long prevalent in European countries was the chief transmitter of the bubonic element of Black Death and that passengers or crew afflicted with plague spread the disease through this flea. The doubts about the presence of rats in Europe from the earlier periods of Christian era having been removed by quoting from history by several writers, it is not very unlikely that the introduction of plague into the European countries might have taken place by the classical maritime ports initially by the infected rodents or their ecto-parasite, resulting in local infection around the port and then its spread into the town and country side in epidemic form. MacArthur (1940) pointed out that according to Geraldus Cambrensis while writing about fifty century, Irish Bishop mentioned that his library was destroyed by *majores mures*, vulgarly called rats. In France, the black rat was certainly prevalent in early 13th century and abundant enough at the time to be a nuisance in England. Evidently, at the time of Black Death (1347-48) during the plague in London and other continental cities and afterwards, the black rat (*R. rattus*) must have been present in sufficient numbers to give rise to serious epidemics, provided that necessary vector flea was simultaneously present. Burnett-Hamilton and Hinton (1916) in their "History of British Mammals" stated that the black rats were firmly established at least after the crusades (1095-1190). Similarly Sambon (1922) who sought for signs of the existence of rats obtained two bronze statuettes of the First century sitein Rome. One of these resembled *R. rattus* and the other *R. norvegicus*. The Romans were therefore acquainted with both species of rats but the skeletons are said to have been engulfed in the lava of the volcanic eruptions of ancient pompeii in A.D. 79. Furthermore, an animal depicted in a Roman tomb of the 2nd centuryat Rheins has been identified as a rat. Other historical accounts are also available, such as that of Gregory of Tours (540-94) who traced the importation of plague from Spain into Marseille harbour in A.D. 588 in atrading ship, to support the view that some species of rodents was the reservoir of infection at the time of the great plague during Justinian reign (542 A.D.). In fact, Sticker (1910b) selected this epidemic from all those recorded by him in the whole course of history as the very type of an outbreak of rat origin. The story of how plague was brought to Byzantine in corn ships from Pelusium, the great commercial entrepot of Egypt, further strengthens the case of involvement of rats. The fossil remains of the species similar to that of black rat have

been found near Geneva, in Lombardy, Bohemia and Crete in Pleiostocene strata. In all probability rats were associated with man since his first cultural beginnings *i.e.*, when he first began to store large quantities of cereals in granariers.

Interrelationship of Rat and Human Plague

When the plague reached Constantinople following Black Death it was accompanied by mortality among rats (Hankin, 1905). Lodge in his treatise 1603 noted that human plague followed when rats, moles and other creatures forsook their holes and habitations and behaved erratically and died. Among the other workers who noted such deaths in the 16th and 17th centuries are Creighton (1891), Hodges (1672) and Skeyne (1688) in Great Britain. During the London plague the belief was generally held that dogs, cats and rats absorbed the plague poison (miasmatic theory) in their fur and could carry it about with them. Defoe went even so much as to say "All possible endeavours were also made to destroy the mice and rats, especially the latter, by laying rat traps and other poisons for them which were numerous in London during plague time, but many of the rats died of the disease and not of poison. Similar observations were made by Loghem (1923) in respect of the plague in the Continental Europe. With reference to plague in France, he mentioned about the triple association of grain storage, rats and plague. The only variety of rat at the time of Marseille plague (1720-1721) was *R. rattus*. Actually rats were mentioned among other domestic animals capable of conserving plague infection. Penalties were imposed on those who threw dead cats, dogs and rats into the streets at plague time, because in all possibility death of rats was due to plague.

In the Eastern countries, the relationship of rats to plague was established much earlier particularly in India and China. The famous Arabian physician Avicenna (980-1037 A.D.) described how rats and other subterranean animals left their burrows and moved about in drunken fashion after the appearance of plague.

The relationship of rats to plague took a more concrete shape in the seventeenth century in India. In 1616 the Treasurer of Jahangir's Court described how handling of dead rats led to plague infection during the plague epidemic in Punjab. Similar observations were made during Dhalawar plague in Central India in 1812 and of the great 'Pali' plague of Rajasthan in 1836. This led to quitting the house or the village at the first instance of rats falling dead, as a preventive measure against plague (Hankin, 1905).

In China, Hung, Liang-Chi (1736-1809; mentioned by Wu Lien Teh) that in the Yun-nan province the plague first affected rats and then human beings. He noted that prior to human plague rats appeared in broad day light and fell dead, and that handling of such rats led to plague in that person. From Yun-nan plague spread to Canton and Hongkong causing heavy mortality among rats and it finally gave rise to the last pandemic passing through Calcutta and Bombay and gradually involving other ports of India,

and eventually other countries.

The knowledge about the role of rats in plague became clearer by the study of Bombay plague in 1895–1898 by the pioneer French epidemiologist, Simond (1898) and also by Hankin (1898) working independently in 1897. Simond drew attention to the way plague was spreading in Bombay. Instead of passing steadily from house to house, the plague appeared in most irregular and unpredictable manner, in houses of different non-connected areas. If more than one person in a household was involved all were affected simultaneously and not successively. Gradually the inmates of another household were infected, who could not possibly have had any contact with or even been near a plague case. Simond observed that plague appeared mostly at places where the number of rats was large. Thus the main breeding places of rats such as granaries were the chief foci of human infection. Both in Bombay and Karachi the first human plague case broke out among the grain dealers. Exactly similar observations were made by the author (Lal and Seal, 1949) in 1948 Calcutta epidemic. Subsequent investigations, of course, led to the full clarification of the situation.

Simond also showed how handling of the dead rats during the Bombay epidemic led to the transfer as of infection to those handling them. Stories of similar episodes were also obtained from Calcutta during 1895-96 epidemic. Simond therefore concluded that plague was primarily a rat disease and that man played a secondary role. Since some cases of plague also occurred in the absence of rats or sometime after the rat mortality ceased Hankin in his 1905 communication postulated that slow development of infectivity occurred in the body of some species of fleas.

In spite of the above important conception of Simond that bubonic plague was primarily and essentially a disease of rats there was a lot of latent period in accepting this hypothesis, as clear links in the chain of infection were still to be revealed. This was evident from the reports of the various National Plague Commissions set up by different Governments between 1897 and 1901 to solve the problem.

The members of these Commissions in their reports did not fully subscribe to the view of Simond as they were still obsessed by the old established orthodox ideas. However, some valuable information was provided by these Commissions. For instance, the Austrian Commission's (1895-1900) conclusion that infection commonly penetrated the surface of the skin and spread via the lymphatics turned out to be of great significance for further probing and there were abundant data on the clinical characters of the disease. As regards the role of rats, this commission took up a cautious attitude. The British Indian Plague Commission (1907-12) from their mass of data contained in their 5 volumes thought that rats were chiefly important for initial outbreak but once established, human agency was considered to be more important for spreading the disease. It was partly true as later observations indicated that man was responsible for transport of rats and fleas. The German Plague Commission (1899) on the other hand, recognized infected

rats as dangerous to man but at the same time concluded that under insanitary conditions plague could flourish without the aid of rats. This was, however, true in case of pneumonic plague. In other words, every commission discovered partial truth, the entire truth was yet to be exposed. In this connection the opinions of two contemporary scientific giants may be quoted. Koch (1898) who visited India in 1897 expressed the opinion that rats were essentially concerned in the spread of plague and further stated that bubonic plague was a disease of rats in which man participated. Sir Patrick Manson also strongly believed that plague was a rat-borne disease and that its prevention lay in the destruction of rats. The above views were also supported by Ogata (1897), Thomson (1900) and Blackmore (1902). Gradually ccnfirmatory evidence accumulated from every quarter of the globe and in the first decade following 1894 multiple observations in Bombay and other parts of the world led to a definite advance from the position that epizootics and epidemics were commonly concurrent to one of human plague dependant on rat plague. Further advances still depended on the clarification of the mode of spread of the disease from rats to man and the link between rat and man.

Seasonal Relationship of Rat and Human Plague

A concerted study to reveal the association of rat and human plague was conducted in Bombay between October 1905 and September 1906 in every sector of the Bombay city (1908). In this investigation distribution of more than 10,000 human plague cases and 117,000 rats, of which 18,000 were found plague infected by dissection, were plotted according to time and space. Five species of rodents collected dead or by trapping, namely, *R. norvegicus*, *R. rattus*, mole rat, bandicoot and mouse were examined. On the basis of gross figures for both live and dead rats, the outdoor rats suffered about twice as severely as the indoor species. In the live rats the rate of infection was 0.85 per cent among *R. norvegicus* as against 0.37 per cent in *R. rattus*, the corresponding figures for dead rats being 22.2 and 16.7 per cent respectively. In one congested area the human plague death rate was 16.4 per 1000 against 1.9 plague deaths in rats per building. On the other hand, areas with fewer plague infected rats per building had the least incidence of human plague cases. The position deteriorated more in March 1906 when 148 out of 296 dead *R. norvegicus* examined were found plague infected *i.e.*, 50 per cent in a single day, and in another group 89 out of 219 dead *R. rattus* were found infected *i.e.*, 40 per cent.

As to the seasonal variation in the incidence of both rat and human plague a well-marked and consistent correlation was noted, which remained consistent from year to year in subsequent observations. In other words, there was a regular plague season usually extending from January to April. In 1905-06 the rise in the outdoor rat falls began in the first week of January and that of human plague in the first week of February. The *R. norvegicus* curve reached the peak in the third week of February and that of *R. rattus*

in the 2nd week of March with a second apex in the middle of April, and a precipitous fall in May (Fig. 5). Occasionally there was a slight revival of the disease during August and September in both rats and man. The hot months of May, June and July were apparently unfavourable for plague.

Thus, *R. norvegicus* curve rises first, followed by that of *R. rattus* and finally of human plague, the interval between the epizootic and the epidemic being about 3 wk. The sequence of events are: Infection by *R. norvegicus* transmitted to house rats *R. rattus*, which in their turn transmit it to man. These seasonal data were analysed further by the eminent British statistician and epidemiologist, Greenwood and his interpretations are given below:

(i) There is an extremely close relationship between the incidence of plague in man and *R. rattus.*

(ii) There is undoubtedly no significant difference between the results obtained by correlating human plague with those of *R. rattus* for the same and preceding four weeks. One value alone approaches the limit.

(iii) The correlation between plague in man and plague in *M. decuminus* is probably spurious depending on the correlation between plague in *decuminus* and in *rattus.* A lag of about a fortnight appears to intervene between the course of *R. rattus* epizootic and human epidemic. The close dependence of human plague and plague in indoor rats is obviously due to intimate association between man and *R. rattus* as in Bombay. However, in Great Britain and European countries where *R. norvegicus* was the common rat, this association was not so close and experience showed between 1900 and 1908 epidemics in East Anglia that the rats in an extensive tract could have been infected without giving rise to more than few sporadic human cases.

Thus the systematic survey by the Plague Research Commission in Bombay and by Thomson (1900) in Sydney (Australia) which received infection from Hongkong and of the epidemic in the same city in 1904 (Thompson, 1905), confirmed once for all the relationship of rat plague with plague in human beings. The time relationship between the epizootic and the epidemic proved even closer than the space relationship which was even better disclosed in this study. A brief account of this study will provide a clear idea about the transmission of infection and the role of rats in human plague. When plague first appeared in Sydney in 1900 the disease was still generally believed to be due to an essentially human intercourse. This was, therefore, the first point that was critically examined by Thomson. Out of a total number of 303 cases examined 289 cases occurred singly in 276 instances and the infection was not communicated to other inmates in spite of ample opportunities. Secondary cases did occur occasionally but it was found on closer study that these resulted from exposure to a common source of infection and not from communication with the sick. Moreover, the hours of attack in the two cases were nearly simultaneous and the cases were of bubonic type. It was, therefore, concluded that some mode of infection external to man should be sought. Further, many of these persons got infected at the centre of the city, which was the original point of invasion, and

retired to their dwellings 2-10 miles away without giving rise to secondary cases.

This observation strongly supported the "localist" theory. But then what was the common source of infection? Thomson noted an unusual mortality in rats which when examined confirmed plague infection by isolation of plague organism. This infection was noted in a wharf prior to the first human case, the diagnosis being confirmed bacteriologically. The discovery of plague bacillus prior to this study was another advantage to establish plague infection in rats and human. Again, outside the body of man plague infection could only be found in diseased or dead rats and human cases continued only as long as rat plague was present somewhere in the neighbourhood. An elaborate study on this point was also included in the study design of the present author (Chapter II).

A more detailed study of the epidemiology of fourth Sydney epidemic of plague was undertaken in 1904 when a clear cut rat epizootic preceded human cases and the epidemic ceased with the cessation of rat death. The search for plague in 1904 having been done with exceptional thoroughness, the correlation in space between rat and human cases became as close as the correlation of time. The ship responsible for the introduction of infection to Sydney from overseas was *S.S. Wyreema* from Queensland with several plague rats on board.

In this connection, Thomson pointed out that search for dead rats required trained personnel with knowledge of the habits of local rats. Actually rats retire to their burrows when epizootic starts and they generally frequent grain godowns, shops and warehouses *etc.* In Colombo epidemic in 1884 few rats were found in the open and the conspicuous evidence of plague epizootic was lacking (Philip and Hirst, 1917).

Role of Animals other than Rodents in the Transmission of Plague

Any death of the farm yard animals at the time of epizootic or epidemic was generally attributed to plague. The Hongkong workers who advocated transmission of disease by ingestion reported ravaging of farm yard stock by plague. Simpson (1905) and Hunter (1904) in association with Matsuda of Japanese Service carried out many experiments on the susceptibility to plague of various domestic animals—calves, hens, turkeys, geese, pigeons, sheep and pigs, both by inoculation and by feeding. Their results were not confirmed by subsequent workers in India (Bannerman and Kapadia, 1908) as well as by the German Commission, except some local reaction in sheep, goats, cows and horses; similar results were also obtained by Watkin-Pitchford (1901) at Natal (South Africa).

Thus farm-yard animals in India and Natal were not found liable to contract plague infection by ingestion, scarification or subcutaneous inoculation. In fact, true plague epizootics do not seem to occur in nature among animals other than rats, mice and some wild rodents. Nevertheless, true plague might sometimes occur in monkeys and squirrels in India as these

animals have been found to be highly susceptible. Plague infected cats are not uncommon in some countries (Araujo, 1937; Moll and O'Leary, 1945) but they are not very susceptible to experimental infection. Dogs and camels have also been suspected (Blare and Baltzard, 1945; Nikanaroff, 1925) but they are rarely affected in nature. Shrews which live side by side with rats being infected with rat ectoparasites may convey infected fleas to human habitations (Sharif and Narasimhan, 1943).

From the above observations it appears that farm-yard and other animals play no significant role in the transmission and maintenance of plague infection, rats and wild rodents being essentially involved. The problem of sylvatic plague actually arose as an issue subsequent to the establishment of rodents as a source of human plague. This will be discussed further in the next Chapter (Chapter V).

CHAPTER V

Phase IV of Evolution of Knowledge on Plague—Discovery of the Causative Organism and Mode of Transmission

Although the basic fundamental question had been answered by the studies carried out during the third phase of the development of knowledge on the problem of plague, a considerable amount of research work was still needed before the necessary epidemiological data were obtained for an effective control of the disease. For even after the discovery of the role of rats and fleas no Government or health authorities of India or any other country could halt the march of epidemics of plague. Prevention of plague thus presented a problem of immense dimension and complexity requiring for its solution a detailed knowledge of the factors governing the interrelationship of man, microbe, rat and flea. Furthermore, no drug had been discovered which could be used for the cure of a patient afflicted with plague. In respect of man, the factors for consideration were the habits of man, his living and socio-culture conditions, the nature of his commerce and communication, his reaction against the infection including his protective mechanism and his role as a reservoir for transmission of infection. For rats and fleas studies were yet to involve their large number of species and characteristics, density of population, behaviour, habit and reproductive patterns and carrier state influenced by climate, harvest, weather and number of other changes in the general environment, their movement, immunological status and change of behaviour during epizootic and many other aspects. In respect of the microbe, complete knowledge about morphological, antigenic, serological characteristics of various types in nature, toxicity, virulence, variability and carrier state which influence the epidemicity and endemicity of the disease was yet lacking. In fact, out of the three essential factors *viz.*, the parasite, the host and transmitting agents only the last factor was partially known till the end of the third phase in the development of knowledge. Finally, the quality of knowledge of the above factors depended on the measures for treatment, prevention and control where only little work had been done.

To an epidemiologist it was of importance to know which factor tended

to increase or decrease plague and how to assess their relative roles in quantitative terms. He also tried to know how many kinds of rats and fleas infest the area concerned, how each species is distributed, their respective habits and relation to man and to one another, relative densities of different species of rats and fleas and infestation in human dwellings, warehouses, cotton and food godowns, *etc.*, their movement pattern, replacement of one species by another and relationship of domestic with wild rodents and perpetuation of infection. It was also necessary to investigate the part played by human agency by introducing rat and rat flea-infested merchandise from one area to another and by ecto-parasites of man and other animals.

Discovery of Yersinia pestis

The discovery of *Yersinia pestis* marked the beginning of phase IV which ended in the middle of the present century covering the peak period of pandemics and epidemics all over the world with attempts to discover effective drugs and antibiotics and antiplague serum for treatment, insecticides for the control of vectors, gaseous and chemical measures for destruction of rats and newer methods of cultivation of plague bacillus for better growth for identification and studies in antigenic structure and development of improved anti-plague vaccine.

Causative Organism of Plague

Towards the latter half of the nineteenth century epidemics of plague were a constant feature of the Yun-nan province of China adjacent to Burma from where it is supposed to have derived the infection (Wu Lien-Teh, 1936). A fuller description of the plague situation in Yun-nan was given by Rocher—a missionary, based on his exploration in 1871 (Simpson, 1905). He attributed the Yun-nan plague to a miasma of the soil first affecting the underground animals such as rats. Lowry (referred by Wu Lien-Teh, 1936), an Imperial Maritime Customs Medical Officer, at Pak-hoi, South China stated that nearly every house where the disease broke out, the rats had been coming out of their holes and dying on the floor.

Until 1894 the progress in plague and occurrence of many epidemics in the interior of China hardly attracted any attention till it reached Canton, and finally Hongkong, the base of the third pandemic. It was Dr Alexander Dennis of the Imperial Maritime Customs, who contemplated the possibility that the disease in man and rat might be identical and due to a microbe and the rodent might be an active agent in transmitting the disease from place to place (Simpson, 1905). Thus the hypothesis originally suggested by Kircher (1638) was about to be fulfilled by the actual discovery of the specific infective microbe in Hongkong.

The new science of Bacteriology having been already established by 1894, when plague appeared in Hongkong, creating an international situation it attracted the attention of the Japanese Government to send a commission to Hongkong. This commission consisted of a bacteriologist, S. Kitasato (pupil

of Dr Koch) and a pathologist, Acyama, and the French Colonial Minister sent Yersin (a pupil of Pasteur) on a similar mission. The former team arrived on June 12, 1894 and immediately set to work in one of the plague hospitals, the latter arrived on the 15th June 1894 and started work soon after a hut was built for him on the grounds of the chief hospital. It is stated that Kitasato received better facilities than Yersin. Both the workers independently discovered the plague bacillus almost simultaneously in the blood, bubo and tissue of the plague patches and isolated it later as pure culture. Kitasato (1894) noticed the organism under microscope on 14th June 1894 and he sent a note of his discovery on the 7th July 1894 to the Superintendent of the Government Hospital at Hongkong and his first preliminary note was published in *Lancet* of August 25, 1894. On the other hand, Yersin's first communication (1894) was read before the Academy of Sciences in Paris on 30th June 1894. He found the organism Gram-negative, which Kitasato had missed, and the organism was pathogenic to mice and guineapig. In fact, Yersin's description of the organism was most accurate while that of Kitasato along with his results of culture in beef tea left doubt in the minds of some bacteriologists. It should, however, be admitted that the technique to isolate the organism in pure culture was not fully developed and it must have been impossible to distinguish *Past. pestis* (now called *Yersinia pestis*) from similar bipolar organism, particularly the spore bearing contaminating type.

Subsequent studies gradually revealed the cultural, biochemical and other antigenic characteristics of the organism and its different types based on virulence and antigenicity (Seal and Mukherji, 1950; Seal, 1940-41, 1942, 1943). At least two distinct races of *Yersinia pestis* could be distinguished by their ability or inability to ferment glycerol; those fermenting glycerol were found in the Northern Asia and in Central Africa suggesting these areas as the ancient endemic foci of plague. The glycerol-negative strains were isolated in India, Ceylon and all those sea ports to and from which the infection had spread during the last pandemic. Both the oceanic and continental races produce nitrous acid from nitrated broth but there is also a South-East Russian strain which is glycerol-positive and nitrous acid negative. Even so, there were technical difficulties in applying the most useful serological test (by agglutinating suspensions of bacilli with blood serum of animals immunised against plague), the bacilli having an awkward tendency to spontaneous clumping. Many workers tried hard to remove this handicap, but they did not fully succeed. The present author, however, took up the work and succeeded not only in removing this handicap by discovering the reasons for such clumping but also prepared a protein free medium (casein hydrolysate) which produced the best and most effective vaccine against plague (Seal, 1943; 1950). The details of the study on the isolation of specific antigen and its utility in the serological and immunological work have been described in Chapter VII.

Causative Organism of Pneumonic Plague

The organism isolated from pneumonic plague cases could not be

distinguished from those of bubonic cases as mentioned earlier (Chapter II). In the opinion of the Indian Plague Commission the primary pneumonic plague might be the result of a mixed infection particularly pneumococal and influenza viral infections. This hypothesis was endorsed by Nicolle and Gobert (1924), Girard (1929) and Sokhey (1950) but no convincing proof could be obtained as otherwise healthy contacts of pneumonic plague cases developed the diseases and died within a short time. Difference in the virulence of the organism was suggested but ruled out by Wu Lien Teh *et al* (1936) according to whom the supposed pneumotropism of *Y. pestis* could develop only through repeated passage from lung to lung and hence does not explain the rise of pneumonic plague outbreaks. Thus no satisfactory explanation could be found for the origin of pneumonic plague.

Role of Certain Factors

Certain wild rodents like *tarabagan* (Siberian marmots) in Russia and Manchuria might account for the frequent appearance of primary pneumonic plague manifestations in the areas in question. In this respect also, no convincing evidence is available but other factors like infectivity of patients, their nutritional status and susceptibility, climatic and social conditions may have some indirect influence. Even so, the role of these factors varies from locality to locality. If adequate facilities for control work are available, outbreaks of this type may be quickly eliminated. Failing such facilities the infection is apt to spread particularly, if there is untoward weather or adverse social conditions, such as, over-crowding or a combination of these factors which were perhaps the causes of devastating plague epidemics and "Black Death" in the mediaeval period in Europe.

Reservoir of Infection

There are at least three kinds of rodents serving as reservoir or hosts of infection namely,

(i) Wild rodents;
(ii) Peri-domestic rodents; and
(iii) Domestic rodents.

Wild Rodents

In trying to trace the gradual evolution of the present day knowledge on the existence and importance of wild rodent plague, the earliest evidence of wild rodent plague was found in the Central Asia where the infection probably originated. This kind of infection among the wild rodents has been mentioned by some as sylvatic plague (George, 1928) and by others as *campestral* (pertaining to level field) plague.

The first statement of the probable prevalence of plague among tarabagan was of a layman, Taherkanoff, who described it in his 'memoirs of a hunter' in Siberia (1856-63; Transbaikalia and Mongolia). This was later also reported in inner Mongolia and confirmed by medical men like Rodenko (1901),

Skchivan (1901) and Barykin (1909). This infection was observed to have spread initially among the hunters and was first identified as plague by Bjeliavski and Rjeshetnikoff (1895). In 1905, the disease was confirmed by actual isolation of *Y. pestis* (Wu Lien-Teh *et al*, 1936 and Barykin, 1909). The Russian Surgeon Rodenko (1901) noted a series of outbreaks among the Cossacks and Nomads dating back to 1887 associated with the capture of silk tarabagans by hunters. He also noted that the disease established in man had a tendency to affect lungs and followed man to man transmission. The tarabagans came out of hibernation in spring. Apparently the infection prevailed among these marmots as natural, independent of man (Pavlovski, 1946). The epizootics among marmots were confirmed in 1921 by Sukonoff (quoted by Wu Lien Teh, 1926) who gave the sequence of events as follows. A person while skinning the carcass of an infected tarabagan either accidentally inoculated himself or was bitten by the infected tarabagan flea-*Oropsylla silantiewi* leading to bubonic plague which then spread to lungs. The great pneumonic plague of 1910-11 sweeping through Manchuria cost 60,000 lives. A recurrence of this episode took place again in 1920-21 killing another 9300 persons.

Y. pestis has also been isolated from marmots in other parts of Central Asia and Southern Kirghis. Besides, several other species of smaller rodents namely, jerboa, suslik and field mice were found naturally infected with plague. Similarly enzootic among wild rodents was detected in the lower Volga in 1878 and in steppes of Southern Russia among wild rodents as well as in domestic mice (Susliks) from 1896 onwards (Nikanaroff, 1926).

The epizootic occurred in summer as well as in autumn for short periods and spread when men came into contact with the infected susliks (squirrels). The plague first triggered off in bubonic form and later in pneumonic form. The fleas involved were numerous (*Suctonophthalmus pullex* and others). The species of wild rodent involved in the maintenances of plague in Iranian Kurdistan was *Mariones meridianus* (Baltazard *et al*, 1952).

In USA human and wild rodent plague infection was detected in 1900 in San Fransisco and by 1910 in other parts of California among ground squirrels (Wherry, 1908; McCoy, 1910; Meyer, 1942). By 1940 wild rodent plague was widely distributed in USA west of Rocky Mountains. The squirrel nests were built in the borrows where food was stored. According to Eskay and Haas (1940) the hibernation period of squirrels was short but they were acting as reservoirs of plague infection. Other wild animals involved were the wood rats of the Southern desert of Nevada (*Neotoma desertorum*) and the white-tailed prairie dogs of the plateau of Arizona and Mexico. Over sixty species of fleas were identified as ectoparasites of these rodents in Western USA. *Oropsylla* (*Diemanus*) *montanus* and *Hoplopsylla anomalous* were the chief fleas found on ground squirrels (*Citellus beechevi*). According to Meyer (1942) many of the rodent species undergo hibernation and aestivation or a combination of both. But such periods of inactivity may not occur regularly or universally as in the case of tarabagan and susliks.

In South Africa human plague was first detected or recognized in the Portuguese territory in 1899 during the Boer war and was supposed to have been imported from plague-infected port of South America through imported grains and forage. Between 1900 and 1905 many South African ports were affected such as Cape Town, Port Elizabeth, East London, Durban *etc.* (Mitchell, 1927). The story was the same for each port *i.e.*, landing of goods followed by rat epizootic in the neighbourhood of the dock followed ultimately by human epidemic. It then spread to other towns and areas through human and other media. By 1903 plague was spread by the infected striped mice (*Rhabdomys pumilis*) from the ports to inland areas till it reached the gerbil colonies of the open field causing widespread enzootics among the wild rodents. Human plague practically died down by 1905 except for small outbreaks in 1907 in King Williams Town and in 1912 in Durban, but in 1914 pneumonic plague followed by bubonic plague was reported in a firm 100 miles inland of East London. Gradually the disease spread to midland districts in Orange Free States and Transvaal. Since importation of infection could not be traced the infection was considered to be of local origin and Mitchell (1927) solved the problem by detecting plague infection among the wild rodents—the gerbils and multimammate mice, and confirming it bacteriologically. There were indications that an extension of enzootic had been smouldering among the gerbils and the field mice of the veld since 1914, possibly since 1901-05. Plague mortality was enormous among veld rodents during the summer of 1934-35. Davis (1946) investigated fresh epidemic of human plague in Bechuana Land Protectorate in 1944-45 following a severe epizootic among the African gerbils of the genus *Tatera*, the multi-mammate mouse (*R. mastomys coucha*) serving as the link between the gerbils in the open veld and man in human dwellings. The main species of flea involved in the African epidemics were found to be *Dinopsyllus lypusus xeridos* and *Chialopsylla rossi D'lypusus*, *X. bantorum* and *X.brasiliensis* and the multimammate mouse. All were efficient transmitters of plague infection to man.

In South America plague seems to have appeared first at the port of Rosario in Argentina in 1899 and spread inland along the transport route by river and rail. Malfran (referred by Moll and O' Leary, 1940) while investigating the outbreak in the Santiago Eastern Province of Chile in 1905 found dead cuises, a kind of wild guineapigs, scattered nearly all over Argentina, but the relationship of these dead cavies with plague was established in 1924 by Uriarte and Gonzales (1924) by isolating *Y. pestis* from two dead cavies. In 1932 an enzootic among them was reported in Cordoba and in 1934 a Commission appointed by the National Hygiene Department in the province of La Pampa described wild rodent plague in Pampa and Rio Negro areas (Barrera, 1937) in which various species and genera of cavies, gallea and caviella were chiefly affected.

Although human cases of wild rodent origin were not common, when cases arose they were generally of pneumonic type. These wild rodents derived the infection from the infected rats of coastal ports. But the plague

among the urban rats persisted in South America longer than in North America and South Africa, suggesting that the rat was the chief reservoir of plague in South America. Knowledge about the species of fleas involved in plague in this country has still remained incomplete. The inter-cavian transmission through cannibalism among them was also suspected. Wild rodent plague was also discovered in Brazil and Andes of Equador. In fact, in the wild rodent plague focus in the Peruvian-Ecuador border region the commensal rats were altogether absent (Macchiavello, 1941a). The human victims contracted plague when harvesting corns in the fields infested with *Cricetinae* which served as a link for passing the infection originally present in tree-squirrels to man. Macchiavello also stated that in Huancabamba (Peru) in the recently discovered plague focus commensal rats were absent and the infection was transmitted by the wild rodent *Cricetinae* invading houses following the harvesting.

Riaz (1948) observed that conditions suitable for exchange of infection between the commensal and wild rodents existed in the Aragua State of Venezuela. There *R. rattus rattus* was often of peridomestic rather than of domestic habits, while *Heteromys anomalous*, one of the wild rodents involved, frequented the fields and granaries and even entered the houses in search of corn.

Peridomestic Rodents

In India, the existence of wild rodent plague foci has not been thoroughly established. From the study of the old endemic foci (Sharif and Narasimhan 1943; 1945) such as the Sub-Himalayan foci in Punjab, UP, north Bihar and particularly in Kumaon and Garhwal, it might appear that the infection was maintained by the wild rodents as expressed by Baltazard *et al* (1952). So far infection has been reported by Simond (1898) in *Funambulus palmarum* in Karachi, *Tatera indica* and *Mus buduga* in the Cumbum Valley of Madras (George and Webster 1934), *Tatera indica* and *Mellardia meltads* in Bombay (Sharif and Narasimhan, 1934), *Tatera indica* in Hyderabad (Rao, 1947) and Uttar Pradesh (Baltazard *et al* 1952) and *Gunomys* virous (*B. bengalensis*) (Lal and Seal, 1949) in Calcutta in 1949. Lesser bandicoot rat-*B. indica* (Indian mole rat) has been gradually invading the city of Bombay and Calcutta and pushing out *R. rattus;* while *B. indica* as fairly susceptible, *R. rattus* is getting resistant (Sokhey and Chitre, 1939). These wild rodents according to their present behaviour can rather be designated as "peridomestic" as they establish relationship with the domestic rats by invading the fields. In all probability these rodents in their endemic foci received infection from importation either by land traffic or by sea routes from elsewhere. In spite of this history and the probability a fundamental question still remains unanswered namely—from where did these commensal rats obtain their infection originally? Since plague is essentially a zoonotic disease of rats it can be safely assumed that it was present in the animal kingdom and after domestication of certain species the infection was brought to human habitation and

since then the cycle is being maintained in certain situations. In case of plague rodents being the reservoir the link between the wild and the commensal rodents are often being served by the peridomestic ones like the *B. bengalensis* in India.

Domestic or Commensal Rodents

From the early history of plague almost all cases of human plague all over the world have been considered to have originated from infected commensal rats. This idea was, however, modified on the discovery of wild rodent plague particularly following the Manchurian outbreak through the marmottarabagan, South African outbreak through the gerbill, American outbreak through ground squirrel and so on. One interesting difference between wild rodent and domestic rodent plague is the pneumotropism of plague organism in wild rodents causing pneumonic plague among men inhabiting the infected areas, while bubonic plague is the commensal type of plague arising from contact with domestic rodents, although occasionally pneumonic type has also been found to arise from bubonic plague accompanied by septicaemia or plague pneumonia; the organism emanating from the respiratory infection then assumes a pneumotropic form.

The commensal rodents implicated in plague outbreaks generally belong to the following three species of Muridae—*Rattus norvegicus*, *R. rattus*, *R. alexandrianus*, and *R. rattus frugivorous* in Egypt. *R. norvegicus*, the predominant species in Europe and America is now being gradually invaded by *R. rattus*. In South Africa and Australia the distribution of rats is similar to that of India. But *R. norvegicus* has been the predominant species in the Latin American countries.

Habits of Common Species of Rodents

Both *R. rattus* and *R. norvegicus* are nocturnal in their habits and spend the day time in their nests where they also accumulate food to provide for emergency. They often go long distance in search of food. They may have intermediary resting places which may be turned into a permanent dwelling in case of need. They move generally along routes mostly close to walls, skirtings, or pipes and keep their teeth sharp by nibbling on wood or in its absence; leather, brick and even cement.

R. *rattus* is a hot climate animal and generally lives in open walls, ceilings and roofs but in cold climate it lives in burrows and shelters. It drinks little and does not like water. Though uncommon the house rat may periodically migrate. The author's personal experience in Assam (Seal *et al* 1951) supports this view. *R. norvegicus* on the other hand, being fond of both hot and cold countries, is hardy for temperate climate, voracious and cunning and adapts itself to all conditions. It is an excellent burrower and swimmer. It drinks freely, is also a diver and inhabits sewers and drains. It has more roving disposition than *R. rattus*, and can undertake lengthy migration with seasonal periodicity. Town rats are comparatively more sedentary in their

habits than rural rats.

Gunomys varius (*Bandicoota bengalensis*) is also a commensal rat in cities or towns and in food stores and godowns. Ordinarily it does not serve as active reservoir or transmitter of plague but during heavy epidemic conditions plague bacilli could be isolated from them also. They will be referred to again while discussing the changing pattern of rats.

Herd Structure of Rodents

(i) *Natural Resistance*

All rodents are not uniformly susceptible to infection. Now a few of the species have been found rather completely resistant to experimental infection with *Y. pestis*. In others there is often marked difference in plague susceptibility. Meyer (1950) found cotton rat (*Sygmodon hispidus*) comparatively resistant, the reticulo-endothelial cells of the liver being instrumental in removing *Y. pestis*. There is also existence of seasonal difference in the species most concerned in the perpetuation of infection. In other words, there is both racial and seasonal resistance though not yet fully elucidated. Meyer (1950 b) also noted seasonal changes in plague susceptibility. A litter of guineapigs amenable to infection during summer was found to become quite resistant during the winter. The present author (Seal, 1960 a) also noted seasonal difference in susceptibility among the local rats. This might be due to climatic conditions exerting influence on the flea vectors and also due to breeding periods. However, the retaining of infection during hibernating period might be due to bacteriophage action (Gaiski, 1944).

(ii) *Acquired Resistance*

A state of non-susceptibility (partial or complete) may develop in individual or groups of rats which have been exposed to plague. Sokhey and Chitre (1937; 1939) using standard test doses examined the resistance of the rats from different cities and towns infected with plague at some time or the other. They found that the percentage rates of resistance were higher in localities which had suffered more plague infestation. Rao (1947) confirmed similar observation in respect of rural areas in Hyderabad State. Sokhey and Chitre (1939) stated that even in localities not visited by plague a minority of rats could be resistant to infection. A point of great importance is how long the resistance becoming manifest in the rat population of infected localities persists in the absence of plague. The findings of the Haffkine Institute (Table 18) show continuity of resistance in high proportion of rats (>90%) although between 1935 and 1939 no plague infected rats were found. One of the reasons for not finding infection in rats was the absence of the methods to test the avirulent or partially avirulent plague strains due to which there was a chance of missing the infection retained by the rat population. The author's laboratory experiment run for 5 yr among the rat population (Seal, 1961) showed continuous existence of such infection and is one

Table 18. Susceptibility of rats to plague in Bombay during the years 1931-1939

Year	Epizootic among rats		Susceptibility of rats to plague	
	Total no. examined	No. found plague infected	No. infected	% mortality
1931	290316	748	118	9.3
1932	272018	600	107	8.5
1933	256900	593	139	7.9
1934	237854	34	93	6.5
1935	226289	0	40	5.0
1936	182727	0	20	0.0
1937	186987	0	90	7.8
1938	187276	0	—	—
1939	207230	0	50	10.0

of the reasons for persistence of infection as a chronic or carrier infection during the interepidemic period. If this condition prevails the rat population may remain resistant for a long period. In case of severe epizootic the entire susceptible lot of rat population is eliminated and in that case few may survive to show resistance for more than a year or so in the absence of any enzootic condition (*i.e.*, plague occurring year after year for some years). Thus in the absence of importation, infection may persist in the subterranean region and may come to the surface even after long periods of absence, as it happened in Calcutta during the last outbreak.

In the normal events in an endemic area an epizootic precedes human cases, first appearing in *R. norvegicus* (particularly in case of imported ones) followed by an epizootic among *R. rattus* and then by human cases (Fig. 22) but during the last Calcutta epidemic (1948-52) the epizootic started among *R. rattus* followed by human cases in Ward 8. But whenever the epizootic extended to a new ward *R. norvegicus* was first affected followed by *R. rattus* and *B. bengalensis*. After eliminating all possible sources of importation it was concluded that the last Calcutta epidemic had arisen out of infection carried over by the partially immunised rats during the interepidemic period.

Diagnosis of Rat Plague

One of the handicaps in the diagnosis of rat plague in the past was the absence of knowledge of the causative organism. The discovery of the plague bacillus by Yersin and Kitasato in 1894 at Hongkong just prior to the Calcutta and Bombay epidemics commencing from 1895-96, opened the opportunity for bacteriological diagnosis of plague infection in man, rat and other animals. Prior to this, for diagnosis of rat plague the Plague Research Commission in Bombay relied principally upon the naked-eye examination of the dissected rats for the presence of bubo, peppered liver, haemorrhage beneath the skin or in the internal organs, pericardial and pleural effusions *etc.* They

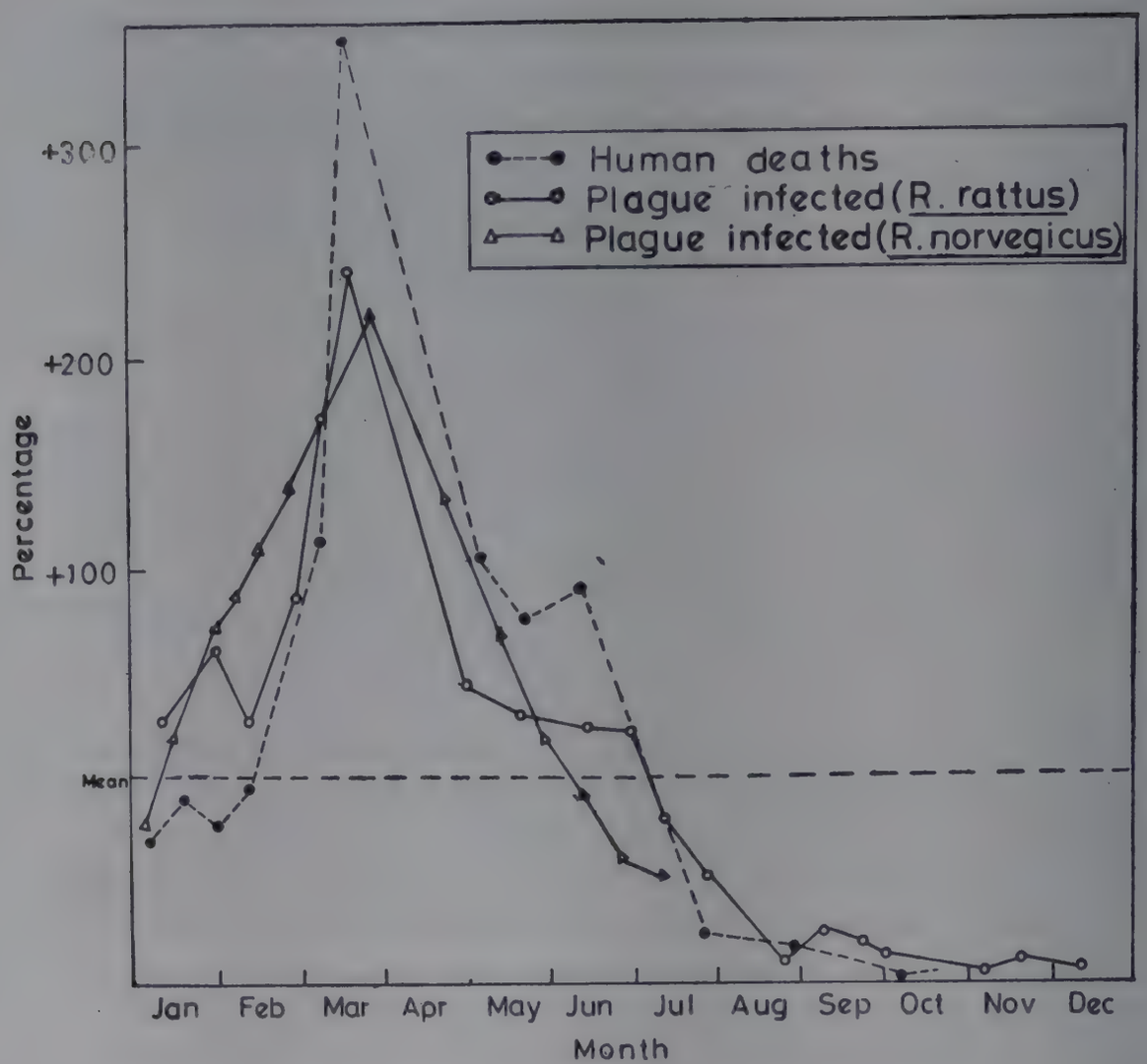

Fig. 22. Percentage above and below the mean relationship epizootic to human case.

considered naked-eye examination as superior to microscopic examination and blood and tissue culture, as in dead rats decomposed tissues were heavily charged with putrefactive organisms some of which resembled plague bacillus. It is true that definite identification of plague bacillus was not possible at that time in the absence of reliable serological as well as cultural techniques. The present author was mainly concerned with the solution of this problem.

It was observed that culture and other tests were also necessary, particularly for identifying infection in sub-clinical and carrier states. In the absence of such a definition culture of tissues (blood, spleen and other tissues) was done in media containing blood, and pooled tissues were inoculated into the culture medium. Even so, the final identification was done by inoculation of such material into guineapigs by scarification of skin or by injecting subcutaneously into the peritoneum. Further improvement of diagnosis of plague infection whether acute, chronic or dormant, by serological, gel precipitation and other tests, has been developed by the present author and described in Chapters VIII and IX.

Mode of Transmission of Plague Infection

Early Studies on the Role of Fleas in Plague Transmission

Although the relationship between rat and human plague was established and the causative germ was defined it still remained to discover the exact mode of transmission of infection from rats to man and rat to rat. Several theories or lines of thought which were developed from time to time explain the mode of transmission are mentioned below:

(*i*) *Alimentary Theory of Plague Transmission*

An influential group of workers consisting of William Hunter, Wilm, Atkinson and Simpson maintained that the plague bacilli were introduced into the body of man or animals by ingestion of infected food. When these animals-rats, cockroaches, flies and other vermins contaminated human food, men ingesting such foods became susceptible to disease. This theory was based on the assumption that plague infected animals—as well as the infected human beings discharge myriads of plague bacilli in the environment through faeces and other discharges. Hunter (1904), the government bacteriologist at Hongkong, claimed to have found plague bacilli in the faeces of man, animals suffering from plague, rendering possible gross infection of food and water, *etc.* Hunter attached particular importance to plague infected cockroaches. Flies and bugs were also found infected. The fact is that in the absence of strict specification for identifying plague bacillus Hunter was dealing with bacilli and not genuine *Y. pestis.* The idea also received support from Simpson's findings of enlarged mesenteric glands, haemorrhagic or swollen coats of stomach congested, the frequency of diarrhoea, vomiting and colic pain at the onset of symptoms of plague in man. The German and the Austrian Commissions working in Bombay also supported this theory of infection by ingestion. Baxter-Tyrie (1905) from his experience of plague in Queensland expressed the opinion that rat to rat infection was possible when the carcass of dead rat was eaten by its commrades. A contrary view was, however, expressed by Simond (1898) and Hankin (1898) and the British Indian Plague Commission (1907).

(*ii*) *Microbes as Contagion*

Following its discovery the plague bacillus was regarded as a communicable contagion in much the same way as the familiar microbic infection-diphtheria. In accordance with this view, the Government of India recommended prompt isolation and segregation of contacts with the sick, the healthy and through disinfection of houses and personal belongings as in ordinary infectious diseases. The government began its antiplague campaign in Bombay with the conviction that the success of control work would depend leaving fewer cases undiscovered (*e.g.*, modern surveillance) *i.e.*, all emphasis was laid upon human factor in plague transmission. Similar idea was also maintained for Calcutta epidemic during the same period. The

report of the Sanitary Commissioner, Government of India in regard to plague was as follows: "Plague is said to be an acute infectious fever caused by a bacillus of Kitasato and Yersin and affecting epidemically and simultaneously lower animals and man *i.e.*, the disease is concurrent and consecutive between man and lower animals (rat), the spread being affected by dejecta and discharges of animals and man. The infection can gain access to the human body via the skin, alimentary tract and by inhalation to the lung". This hypothesis assumed that plague bacilli were freely discharged from bodies of infected men or animals, that they survived for an appreciable period in external environment and were commonly found on the surface of soil, on the floors of houses and on other objects in the infected locality including food-stuff.

A few years later the same Plague Commission showed that all these assumptions were untenable. Hankin (1898), Simond (1898) and few others pointed out that plague bacilli could survive only for a short time outside the body at tropical temperature and could scarcely be recovered from the objects supposed to convey the infection. However, the Bombay Health Authorities used disinfectants on a gigantic scale by employing a staff of 30,966 persons. Disinfectants were pumped into sewers at the rate of 3 million gallons a day. Much clothing and many suspect objects were burnt including even suspccted grains. The result was, however, contrary to expectation. The method drove away infected rats more into human dwelling resulting in more cases in the neighbourhood and at distant places.

By 1897, it became clear by observation and study that typical bubonic plague, though due to a bacterium, could not be regarded as infectious in the ordinary sense. Only when pneumonia occurred as a secondary complication that the possibility of man to man transfer was greatly facilitated. Hence hospital authorities had to take precaution by strict means of isolation.

(*iii*) *Microbes and Miasma*

The discovery of the specific micro-organism of plague undermined the position of the supporters of miasmatic theory. But the interesting story is that the belief that plague was due to pollution of air was widespread in the early months of Bombay epidemic in 1896, and that the disease was spread by currents of polluted air emanating from the granaries of the Port-Trust. It was equally fashionable to believe that free ventilation and exposure to fresh air was a safeguard against plague. For instance, it was noted that mendicants living in open air were least affected. The idea was supported by Thomsons in the "Treatise on Plague" published in 1901, following their experience of plague in the Satara District in 1897-98. Apparently this theory supported the idea that the virulence of the germ is neutralised by fresh air and the resistance of the people is also enhanced thereby. Latham (1900) also attempted to reconcile microbes with miasma by pleading the case of seasonal prevalence, the cases rising to maximum when the ground exhala-

tions were greatest, releasing larger number of plague bacilli from the soil. We now know that seeds of infection do not lie in the interstices of the soil but in the bodies of animals living in the subterranean burrows.

The above hypotheses failed to establish the exact mode of transmission and several questions still remained unanswered in the face of the following observations by Simond (1898).

a) Plague in man as well as in rat was found to be interrelated, but the mode of transmission of infection was not known.

b) Goods like grains, raw cotton and clothing were found to be vehicles of transport of plague infection though bacteriologists could not find plague infection on them. This phenomenon could not be explained.

c) When a person suffering from plague arrived at an uninfected place he was not found to infect any other person. It was surprising as to why no secondary cases arose although the person was a veritable depot of infection.

d) Even when importation of a plague case in an uninfected place was followed by an outbreak of the disease, there was a latent period of few weeks that frequently intervened between the arrival of a case and definite outbreak of the disease among the local inhabitants. This lag could not be explained.

e) Cases usually occurred among those who handled dead rats but such danger was apprehended on handling the carcass left out for a few hours. The reason for this could not be ascertained.

f) Infection was noted to persist in some localities after all rats or all signs of rat plague had apparently disappeared from the scene. The phenomenon could not be explained.

The experiments of Simond (1894) in Bombay provided the clue to many an unanswered question. Simond observed that plague was not easily transmitted among rats by ingestion of infected food or by mere contact with heavily infected material. However, rodents could be easily infected by pricking their skin with a needle infected with plague bacilli. Placing these bacilli on intact rodent skin did not infect them. Simond therefore argued that a definite breach in the intact skin (epithelium) was necessary to enable *Y. pestis* to penetrate into the body of the animal (or man) to produce the disease. The essential point was that in both rat and man common mechanism must be existing by which the plague germs could be inoculated through the skin by puncture. This mechanism could be the bite of a suctorial insect in both rat and man. Thus the transmission of infection by the bite of an infected rat flea seemed to be the most promising hypothesis. Bed bugs were also thought of transmitting the infection in case of man to man transmission. The observations and experiments carried out in succession by Simond to provide convincing proof of his theory are briefly described below:

(i) As a first step, he looked for evidence of a definite point of entry of plague bacilli on the surface of the skin of a patient consistent with the flea bite. He found phlyctenule a characteristic local lesion in 5 per cent of

plague cases. He carefully avoided the possibility of this lesion being due to any accident. Examination of these phlyctenules bacteriologically yielded *Y. pestis*. This finding was supported by chance infection of Dr George Sticker, a member of the German Plague Commission who accidentally got pricked while carrying out a post-mortem examination on a patient who died of plague. He developed a phlyctenule in his right thumb followed by a bubo in his right arm but fortunately he survived. Two similar cases occurred among the members of the Japanese Mission at Hongkong in 1894. The evidence of entry of plague bacilli was not present in most of the cases but the cases in which it was present generally survived indicating some resistance. The site of bite and of the phlyctenule was on soft part of the foot in the neighbourhood of the ankle and not on the sole or side of the foot. Therefore, it seemed clear that some active agent must have introduced the infection beneath the skin and in all probability the agent was blood sucking ecto-parasite.

(ii) The next point that arose was to ascertain whether fleas, the ecto-parasites of rats, could actually bite man. On getting positive results his next attempt was to establish that fleas taken out of infected rats contained *Y. pestis*. He confirmed this by microscopical examination of the fleas and crushed material from these fleas. Unfortunately he did not have the facilities for cultivating the organism to be sure of the specific identity. He, however, obtained an indirect proof that fleas taken out of healthy rats did not show any similar organism.

(iii) As a next step the fleas taken out of healthy rats were fed on a plague infected rat and after being sure by examining some of them 24 hours later that they had imbibed the infection, a group of three mice were inoculated with an extract of the infected fleas in water. All the three mice died; however, only one of them showed plague bacilli.

(iv) The next step was to carry out a natural experiment with infected rats to confirm the results of the artificial experiment. He took a sick rat captured from infected premises and placed it in a large glass jar together with twenty cat fleas (at that time he had no idea of the efficiency of fleas to imbibe and transmit *Y. pestis*) to supplement them on the body of the infected rat. When the infected rat was about to die (25 h later) a small iron cage containing a young rat with grill open on one side only was inserted into the jar. The sick rat died a few hours later and the carcass withdrawn 36 h later. The post-mortem disclosed plague bacilli. The young rat that remained in the jar exposed to the infected fleas eventually became sick and died on the fifth day with lesions typical of plague and showing plague bacilli in the smears. The experiment was repeated thrice with one success and two failures. The question not understood well at that time was that the adult rat captured from the locality might have been relatively immune. Further, the flea—*Stenocephalide felis* used was very inefficient vector of plague.

On the basis of close epidemiological reasoning combined with the above

observations and experiments, Simond reached the vitally important conclusion that fleas were primarily concerned with the transmission of plague infection in both rats and man and thus provided the missing link. He had carried out these experiments with many handicaps namely, his laboratory was only an improvised camp having no facilities for culturing the organism. Also he had no good knowledge of entomology and of rodent characteristics. He therefore realized that his hypothesis would raise a number of questions requiring solution. For instance, he observed during experiment that fleas while feeding blood also defecated and this led to the suggestion that plague-infected faeces might be inoculated into the wound made by proboscis of the fleas. This was, however, later confirmed by Bacot and Martin (1914) in their experiment.

Another suggestion made by Simond was that fleas kept in test tubes in the laboratory survive only a few days but in nature these infected fleas may live long enough to infect persons sometimes even after the rats have disappeared or died out. Secondly, variations in the number of fleas according to local conditions might play an important role in determining the severity of an epidemic and afford a solution of the problem of plague recrudescence. Thus, later on, it was proved that the flea-index was an important element in the epidemicity of plague. In fact, Simond's ectoparasitic theory of transmission of plague infection (though weakened by some experimental drawbacks) provided the answers to the majority of questions initiated at the onset of this chapter. Thus, a) In the absence of ectoparasites infection was not transmitted by cannibals or by contact with infected matter of cadavers; b) It explained why it was dangerous to handle carcasses of infected rats and infected ectoparasites seeking new hosts to feed; c) It explained why bubonic plague cases did not transmit infection to contacts. It needed an ectoparasite. Besides, these ectoparasites could imbibe infection only when there was a great number of bacilli in the blood stream which a biting flea can suck. This was not possible ordinarily in bubonic cases; d) It explained why ordinary disinfectants failed to control the disease.

Thus Simond's work justifiably provided a solid basis for further investigation and was helpful in providing explanation for preventive work with DDT and not by bactericidal disinfectants.

As is generally the fate of all new discoveries or theories this new conception of bubonic plague as a flea-borne disease was opposed by many epidemiologists and various plague commissions. The British Indian Plague Commission rejected the transmission of plague by puncture of epidermis by blood sucking insects on the basis of weak evidence produced by Simond. According to them the rapid decline in the infectivity of dead rats was attributable to rapid fall in the number of viable plague bacilli in the rat carcasses. Yet the Commission thought that man was usually infected by microbes exposed on the surface of the ground. The German Plague Commission rejected the ectoparasitic theory on the plea that hospitals abound in these parasites and yet transmission does not take place. They thought that clima-

tic conditions prevailing in Bombay (cold weather) and lack of personal hygiene favoured multiplication of parasites. On the other hand, both the Austrian and Russian Commissions agreed with the cutaneous transmission of infection, without giving a satisfactory explanation of the modus operandi. Hunter (1904) and Simpson (1905) failed to infect rats with infected rat fleas. Kolle (1901), Klein (1905) and Nuttall (1899) also failed in the transmission experiments. The latter was inclined to attach some importance to infected fleas having found the organisms in fleas feeding on plague infected dead animals (but there is no epidemiological evidence that fleas play any role in plague epidemic). Recent research, however, shows that lengthy interval (as long as even 15 days) may elapse between the time of infection of a flea and the time it is able to transmit plague infection explaining partially the cause of failure of the above authors.

In any case, there is no doubt that Simond was able to establish a strong *prima facie* case for further experiments to prove his point. There were some workers such as Thomson (1905) and Ogata (1897) of the Hygiene Institute of Tokyo, Japan, who supported his theory. In his paper on Formosa Plague in 1896, Ogata suggested that rat fleas might be implicated in the spread of plague, thus anticipating Simond's observations after a few months. He surmised that rat fleas might attack man after deserting the cooling body of dead plague rats. He was perhaps the first to suggest that fleas might play a part in the spread of plague, though assigning no leading role to it. Tidswell (1903) of Sydney also supported Simond and used *X. cheopis* for transmission. Zirolia (1902) of Rome found *Y. pestis* to survive for 8 days in the stomach of fleas. Gauthier and Raybaud (1903) successfully repeated Simond's experiments working in Marseille. The transmission cage employed by them was divided by a pair of grid in the centre into two compartments about one inch apart, so that rats were kept apart but fleas might pass freely from one compartment to the other. There was still one loophole left and that was the possibility of the parasite (infection) being transmitted through air. This was obviated by the experiment carried out by Verjbitski in 1908. He placed a glass wall between the two sides of the transmission chamber to prevent any infection through air. This barrier was removed when the infected rat died. The success of the experiment, however, depended on the choice of the type and species of the flea employed.

Final Proof of Plague being a Flea-borne Disease

A careful epidemiological field investigation and analysis of the results of observation of bubonic plague cases in Australia by Thomson (1905), Tidswell (1903) and Ham (1907) and of the epidemiological-cum-field experimental observation of Liston (1908) at Bombay finally justified the conclusion that rat plague is flea borne, and that it cannot be transmitted continuously as an epizootic in the absence of rat fleas. From the study of plague in Sydney, Thomson developed a hypothesis that living mobile agent operating independently of man, must be primarily concerned with the spread of

the disease and this agent was the rat. But the question was how this infection was communicated to human beings. The ingestion theory of Hunter was not operating in Sydney as the infected persons brought their food in a closed packet from home and it was opened at the time of consumption. On the other hand, the pathological studies carried out in Bombay showed that the route of infection was through the lymphatic channels *i.e.*, inoculated directly through the skin. Tidswell confirmed this Bombay finding. Two things were therefore necessary namely, a breach in the skin and introduction of freshly deposited organism into that breach. It was quite easy for the barefooted Indian to get such infection but how it became possible for the Australians wearing boots to get it. Lawson (1897) however, pointed out that soldiers in India who wore boots also got plague. Careful analysis of 12 cases who had developed solitary buboes in 1902 epidemic indicated that the infection might have been initiated while wearing boots. In fact, in the first case of plague he discovered a phlyctenule in the leg, of the type described by Simond. This led to the suggestion that some living intermediary existed between rat and man which explained a number of epidemiological anomalies namely, persistence of infection after all evidence of rat plague had disappeared from the premises; presence of rat plague in some houses without any human plague; and the fact that rats which died of plague were harmless to man for sometime.

The rat-flea was thus considered as the missing live intermediary. Moreover, the season of highest human and rat plague incidence was also the season of greatest abundance of rat fleas in Australia, particularly that of *Pulex pallidus* (same as *X. cheopis*). The ecto-parasites are generally host-specific. The question was raised as to how the flea can bite man? Liston (1908) provided evidence to show that under natural conditions the rat fleas of the city readily attacked both man and guineapigs when they were hungry and their normal host rats were not available.

In March 1903, while Liston was in charge of the Plague Laboratory in Bombay, he received some guineapigs which had died of plague, from the Zoological Gardens. On local investigation he found the surviving guineapigs covered with rat fleas (*X. cheopis*), the sick guineapigs being covered more than normal ones. Normally guineapigs are not flea-infested. In April 1903, Liston received some fleas caught on man in a plague-infected house with history of rat deaths about a fortnight earlier. After rat falls had ceased the inhabitants were so much troubled by such large number of fleas that they were obliged to quit the premises and sleep outside, and within a short time two persons developed plague. Fourteen of 30 fleas caught were *X. cheopis*. These observations led Liston to conclude that when rats have died of plague or have deserted the permises on account of high mortality among them the infected fleas after a few day's starvation came out in the open in search of new hosts and then readily bite man. On the basis of above experience Liston decided to use healthy guineapigs to be used as traps for infected fleas by placing them in rooms where rats or men have died of

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plague. This new technique proved successful. Not only he could collect numerous fleas on the body of these guineapigs, he found some of them to have plague bacilli also. Some of the guineapigs eventually died and numerous plague bacilli were isolated from the carcass. As a control study, he placed some guineapigs in healthy houses. They neither attracted fleas nor any of them died of plague. To this day it is the practice to use sentinel guineapigs for detection of plague infection in the suspected houses.

Liston (1906-12) pointed out that plague was most prevalent in places where rat flea infestation tended to be high, and the men living in dark and dusty habitations of working in granaries and places where fleas breed freely were most prone to plague, whereas those who were in the habit of anointing their bodies with oily substances deterrants to fleas were least subjected to plague.

Species of Fleas in the Transmission of Plague Infection

The next obstacle in establishing the mechanism of transmission of plague infection was the failure of certain fleas to transmit infection experimentally. The observations of Tidswell (1903) on Australian rat flea opened a new and important chapter in the history of epidemiology of plague. For the first time the flea-species factor and the relative distribution of different species of rat fleas on the spread of the disease in space and time began to attract attention. The position has been well-defined by Ham (1907) in his report on "Plague in Queensland during 1900-07".

Plague infection was introduced into Brisbane (Queensland) in 1900. Ham was impressed by the occurrence of plague in situations where the strictest search failed to reveal the presenee of sick or healthy rats, but where straw, chaff and other packing materials were present. In at least five instances such material was found which was obtained from produce stores in which plague infected rats had been previously discovered. Fleas collected from these materials were found plague infected pointing out not only to the survival of plague infected fleas after the death of their hosts, but also to their passive transfer from the place where they became infected to a distant locality giving rise to a new outbreak of plague. The spread of plague by flea infested produce turned out to be of prime epidemiological importance.

Seasonal Prevalence of Plague

In regard to the marked seasonal variations in the incidence of plague among rats noted in Queensland and other parts of Australia, Ham (1907) observed that "more feasible explanation of the seasonal variation might be found in the prevalence or activity of insect life, notably fleas, and the development of the egg, larva, and pupa of fleas under certain environmental conditions. Actually he found an increased prevalence of fleas in the months of January and February in 1907 during epidemic of plague, which became scarce during the off season. This seasonal behaviour was later confirmed by Bacot and Martin (1934), the distinguished entomologists. Ham even

suggested that meteorological variations might have a seasonal effect on survival, growth and virulence of the bacterial flora, including the plague organism. Still more significant was Ham's observation on the regional distribution of the different species of rat fleas in relation to plague, *X. cheopis* being the most prevalent rat flea in Australia (90.8% out of 1609 rat flea studied). Subsequent rat flea survey showed that New Zealand and Tasmania were free both from plague and *X. cheopis*. *N. fasciatus*, a non-vector of plague for all practical purposes, was present in the above two islands whereas the rats of Sydney, Brisbane, Rockhampton, Townsville *etc.* showed a heavy *X. cheopis* infestation and their heavy seasonal prevalence corresponded the plague season. Experimentally also, *N. fasciatus* and *L. segnis* proved to have poor aptitude for human blood. On the other hand, the relative distribution of the various species of rats did not appear to be a factor of great importance in the epidemiology of plague in Australia judging from the data given in the official history of Australian plague.

Further Experiments in Bombay

Following the pioneer investigations mentioned above the Commission (1906-12) for the investigation of plague in India (Bombay) headed by Sir Charles Martin and having Drs Lamb, Glen Liston and Gloster of the Indian Medical Services and Drs Rowland and Petrie from the Lister Institute of Preventive Medicine and some Indian staff planned in 1905 to carry out thorough testing of the hypothesis that plague was a flea borne disease. The team worked in the Parel Laboratory, Bombay till 1910. Four series of experiments were performed with fleas taken out of rats found in Bombay particularly *R. rattus*. In the first series healthy rats and infected rats were kept in close proximity but not in contact; in the second, fleas taken off rats with septicaemic plague were transferred to healthy rats in the flea proof cages; the third experiment was to ascertain whether a plague epizootic could be maintained among rodents by means of rat fleas in specially built cabins simulating conditions found in nature and whether such an epizootic could proceed in the absence of any flea; and the last series was conducted with rat fleas captured from the infected premises in the Bombay city.

The results of these experiments were summarised as follows:

(i) When the fleas were kept off the cabins plague never spread among the healthy animals however close and prolonged was the contact between them and the animals died of or suffering from plague though the cabins were not cleaned out and the food was supposed to be contaminated with excreta.

(ii) Even the new-born pups sucking infected mothers did not become infected, if fleas were completely excluded.

(iii) Once the fleas in the huts were infected by introducing plague infected rats into the herd a continuous epizootic prevailed till all the rats died or the number of fleas were exhausted.

(iv) If infected fleas were entered into the protected cabins previously

free from fleas the epizootic started among healthy rodents, which prevailed as long as the supply of fleas was maintained.

(v) Monkeys or guineapigs kept in cages 2 feet above the ground and protected from fleas by tanglefoot around the cages remained unaffected but if the cages were lowered down at a height that a flea can jump and no tanglefoot was placed they were readily infected and died of plague. This excluded the possibility of aerial infection.

(vi) In the cabin experiments the wild live *R. rattus* were given the opportunity to eat the carcass of rats died due to plague. None of them got plague after being exposed to infection by mouth, when fleas were completely excluded.

(vii) In the last set of experiments healthy guineapigs free from any fleas were allowed to move about freely in plague infected houses. After some time numerous fleas could be captured on the body of the guineapigs (as many as 263, mostly rat fleas in a single room).

Many of these fleas showed abundant plague bacilli in their stomach and many of the guineapigs used for trapping them died of plague (this happened in spite of the floors of these rooms being disinfected with bactericidal disinfectant, mercuric perchloride, indicating the uselessness of such disinfectants).

Against this observation, guineapigs kept in cages protected by tanglefoot remained healthy even in the most heavily infected premises. The fleas recovered from the guineapigs from infected houses could transmit the disease to healthy animals in the same way as the fleas recovered from dead or dying rats.

Thus the above experiments gave the final and conclusive proof of the role of fleas in the transmission of plague infection from rat to rat and rat to man. In fact, the period between 1894 (the year of discovery of plague bacillus) and 1908 (when it was proved that plague is transmitted by rat fleas) may be termed as the "Pioneer Phase" of plague researeh in the early years of this century.

Even so, knowledge did not yet reach a stage of its end. Many things were still unknown to us for further or continuous study with many new situations or conditions arising as time passed on.

Further Progress in Knowledge about the Role of Fleas in the Transmission of Infection

Though the basic question had been answered on the role of fleas, the ectoparasites of rats, in the transmission of plague infection from rat to rat and rat to man, an immense amount of research work was still needed to be done before the epidemiological data for the effective control of the spread of the disease could be obtained. In fact, the progress of plague is affected by a large number of ecological conditions *e.g.*, habits of man, the return of his commerce and communication, density of population and habits of different species of rodents and fleas, as influenced by climate, weather,

harvest and a number of other changes in the general environment. The taxonomy of the Siphonoptera to which the fleas belong was in a rudimentary stage when Simond, Ogata and other pioneers first began to realize that the bubonic plague was a flea-borne disease. The knowledge has since been improved by the work of Rothchild (1903) who gave the first detailed description of *Xenopsylla cheopis* under the name *Pulex cheopis* (Syn. *murinus* and *pallidus*). In 1914, he showed that there were three Indian rat fleas belonging to the genus *Xeopis viz.*, *X. cheopis*, *X. astia* and *X. brasiliensis*, and that epidemiologists could easily differentiate them (Rothchild, 1914). In 1927, Hirst stated that *X. cheopis* was the paramount species found on plague rats throughout the world, *X. brasiliensis* was next in the rank and the vector-efficiency of *X. astia* was poor. Rats harbouring *X. astia* alone, as in South India and Ceylon, were seldom affected with plague. In the temperate climate *Xenopsylla* species of fleas was found to be replaced by *Nosopsyllus*, *Stivalius* and *Leptopsyllus* at the highest elevation of mountains in Ceylon, Madras, Java and the Himalayas.

In general, fleas of the genus *Ceratopyllus* predominate on the rats of temperate regions, *e.g.*, *Ceratophyllus fasciatus* in Great Britain. This species has been differently named according to different local areas *e.g.*, *N. punjabensis* (in Punjab), *N. nilgiriensis* (in southern India), *N. tamilnus* (in Ceylon), *N. anisus* (in Japan) and *N. nicanus* (in China). These fleas act only as passive reservoirs of plague infection in the rat's nests and never or seldom play any significant part in the spread of human plague. Similarly *Leptopsylla segnis schonherr*, although widely distributed on rats in cooler parts of the earth, seldom bites man is an indifferent carrier of plague to rats. *Pulex irritans*, the human flea has been suspected of transmitting plague from man to man.

Interchange of Fleas between Commensal and Wild Rodents

In the western areas of USA several early observers (noted by Prince, 1943) had found ground squirrel's fleas on commensal rats. That the flea involved was mainly *D. montana* was confirmed by Meyer and Holdenried (1949). The subspecies flea *Rhopalopsyllus* was found on both field and wild rodents in Venezuela (Hecht, 1942), Peru and Ecuador (Ramos Diaz, 1938), Belgian Congo (Devignat, 1946) and Kenya (Heisch, 1952). *X. astia* was for sometime found in semi-domestic rodents in India (George and Timothy, 1941).

In the steppes of South-East Russia a free exchange of ectoparasites seemed to have occurred among all the miscellaneous rodents including the susliks, field mice, jerboas and gerbils, who all shared the general epizootic (Nikanoroff, 1926) to some extent. Thus, the interchange of fleas between commensal and wild rodents was not uncommon, but such fleas were generally poor vectors.

Longevity of Fleas under Different Environmental Conditions

There are four stages in the life cycle of fleas, namely, the egg, larva, pupa and adult. With optimal climatic and biological conditions, the life cycle can be completed within 3 weeks. Bacot (1914) observed that fleas could live independently in the burrows for a considerable period (10 to 22 months) depending on the species. Macchiavello (1947) observed in Peru that *X. cheopis* could remain infected in empty burrows for periods up to six months or even longer. George and Webster (1934) while experimenting with wandering fleas (*X. cheopis* and *X. astia*) found that the fleas were capable of transmitting plague after periods of starvation up to 29 days. The importance of free living fleas was also shown by Girard and Estrade (1934) and Robic (1937). Wild rodent fleas could survive for prolonged periods in burrows under hibernating condition. For instance, suslik flea could stand low temperature up to 25°C and prolonged starvation up to 10 months (Golov and Ioff, 1926). Large scale investigations carried out later by Tiflow and Ioff (1932) showed that fleas of south-east Russian wild rodents kept starving in a moist atmosphere and could survive at room temperature up to 292 days and in the cellar up to 369 days. If fed periodically they could survive for a maximum period of 1725 days in the case of female *Neopsylla setosa* (females were hardier than males). Burroughs (1953) who carried out extensive experiments on the longevity of both wild and commensal fleas concluded that wild rodent fleas live longer (182-477 days) than house rodent fleas (158-281 days) when fed daily at a relative humidity lower than 90 per cent. They survived for 17-52 days when fed only once. Nicoll (1912), Bacot and Martin (1914), Goyle (1928) and Brooks (1917) concluded that high saturation deficiency and high temperature exerted an adverse influence on adult fleas and that microclimate in burrows was more suitable than outside temperature and darkness was preferable to light. Other conditions of growth of larvae and adults have also been studied. According to Webster and Chitre (1930 a and b) high temperature above 95°F is not conducive to transmission of plague infection.

Rat Flea Density (Flea Index)

Studies made in India and Australia at the beginning of the century indicated the importance of rat flea density or flea-index. In a given number of rats the number of fleas per rat determined the ease with which the infection spread from rat to rat and rat to man. This was also corroborated in experimental rat epizootic. High rat and rat flea densities were found to be closely correlated (Steenis, 1923; Hirst; 1927 and Eskey, 1934). The average number of each species of fleas found on the bodies of each species of rat was the figure commonly reported (specific flea index), the *Cheopis* index being the most significant figure if that flea was present. Now-a-days the practice is not only to estimate the specific rat flea index but also to estimate the fleas in the rats' nests in burrows as well as those wandering freely. Nevertheless, it is the fleas leaving the cold bodies of rats dead of plague

which are important. Some of the fleas recovered from burrows may be female fleas retired there for laying eggs. According to Kolle (1945) temperature also exerted an interesting effect on the sex distribution of rats and fleas, the male rat predominating on warm days and the female flea on cold days and since the female counts are less variable than the male he suggested that flea indices should be based on counting females only.

X. cheopis is the flea which infects *R. rattus* and *R. norvegicus*. It is essentially an indoor flea and hence high cheopis count indicates greater danger of spread of plague. While quoting flea indices it is also essential to quote the type of rats. In fact, Webster and Chitre (1930 a and b) found high percentage of *X. cheopis* on in oor rats and that of *X. astia* mainly on outdoor rats—*Gunomys kok* (*B. bengalensis*) and *R. norvegicus*, and gerbils (*Tatera indica*). *X. brasiliensis* is less of an indoor flea than *X. cheopis*, and is common in and around the huts of natives of Central Africa.

Absolute Flea-Index vs Specific Flea Index

The earlier workers depended on the collection of fleas from rodents trapped alive, taking each rodent species separately. The specific flea-index is the average number of fleas of each species found per rodent and the total flea index is the average number of fleas of all species per rodent. Some workers (Davis, 1939 and Macchiavello, 1948) recommended the determination of total or absolute flea index which includes the results of examination of burrows, nests and free fleas. Various methods have been suggested by different workers on collection of free fleas or those from burrows and nests. In the opinion of some workers more reliable results could be obtained in flea survey by computing the percentage incidence of the various species rather than by finding indices. According to King and Pandit (1931) it was best to determine both the specific and per cent indices of the various flea species in order to obtain fully reliable results. Hist (1926) and Grubbs (1927) concluded that if *X. cheopis* index was kept below 1, there was no danger of an epidemic. This was also true if the cheopis percentage was below 20-30 per cent or the cheopis infestation rate was below 30 per cent (Eskey, 1934). Webster and Chitre (1930) however, maintained that the plague season need not correspond with the season of maximum prevalence of rat fleas although this could happen as a coincidence and that the proportion of the infective fleas was important.

Mechanism of Plague Transmission by Fleas

After it was settled that transmission of infection from rat to rat and rat to man took place through fleas, various theories were advanced and experiments performed on the actual mechanisms of transmission. Four modes of transmission described below were contemplated and experimentally tried by different investigators.

Transmission by Faeces

Transmission of plague infection by faeces of fleas was based on the

finding that fleas excreted plague bacilli in their faeces and some of the observers noted survival of plague infection for prolonged periods even in dry faeces. Swellengrebel (1913), however noted that *X. cheopis*, *N. fasciatus* and several other fleas did not usually defaecate while feeding and that the faeces adhered to the fur. Burroughs (1947) confirmed the above findings. Wheeler and Douglas (1943) and Golov and Ioff (1926) noted very irregular excretion of plague bacilli in faeces and Eskey and Haas (1940) failed to transmit infection in guineapigs by cutaneous route but succeeded in doing so by intracutaneous inoculation. Based on the above findings the probability of transmission of infection by faeces could not be fully supported.

Transmission by Oral Route in Rats

Since gastro-intestinal type of infection is practically absent in naturally infected rats, the mode of infection was considered most unlikely by ingesting either faeces or infected fleas but there are records of transmission of infection by consuming the carcass of rats died of acute plague.

Mechanical Transmission

The Indian Plague Research Commission while working with fleas *en masse* which had been fed on infected rats, obtained positive transmission within 36 h. But these infected fleas continued to be infective even a few days after feeding on infected rats and thus went against the hypothesis of mechanical transmission. This was further confirmed by experimenting with individual fleas by Burroughs (1947). On the other hand, it was possible to transmit the infection by exposing the laboratory rats to the bites of numerous *X. cheopis* and *P. irritans* in less than 24 hours of their leaving the dead hosts (Blare and Baltzard, 1945; Burroughs, 1947). Analogous experiments with wild rodents were frequently negative. Burroughs therefore thought that all fleas were not efficient transmitters and that there must be some special mechanism which made certain fleas more efficient transmitters than others. Accordingly the success of transmission in the above *en masse* transmission experiment indicates that mechanical transmission could perhaps be effective during heavy epizootics through frequent bites to the new hosts while viable plague organisms still remained in their mouth parts.

Transmission by "Blocked" Fleas

The mechanisms stated above did not seem to be a common or regular method of transmission. Another possibility made out by the Indian Plague Research Commission was that a regurgitation of stomach contents of plague infected fleas might play a role in the transmission of infection. The question was solved by Bacot and Martin (1914). They showed that rat fleas could often infect by direct bite under close observation, and that such fleas exhibited characteristic symptoms and signs due to blockage of the gizzard or proventriculus with plague growth so that the thirsty insect was unable to suck blood into the stomach but regurgitated it by elaslic recoil of its

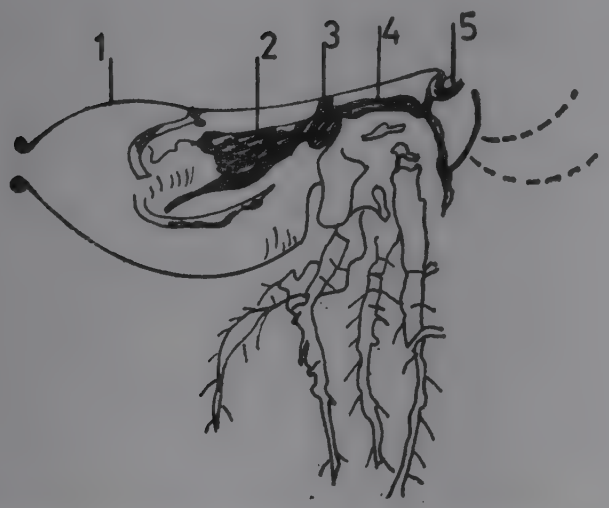

Fig. 23. Blocked art flea. 1. Masses of *P. pestis* 2. Stomach 3. Proyentricular value blocked 4. Ogsophagus with masses of *P. pestis* 5. Phyaryngeal pump.

distended oesophagus and pharynx back into the wound made by its proboscis together with masses of plague bacilli detached from the obstructing growth (Fig. 23). They therefore concluded that this was the typical mechanism by which the insects transmitted plague. The "blockage" phenomenon could be seen under the dissecting microscope or hand lens. It was further observed that a passage might be cleared through the centre of the mass of plague bacilli by autolysis, leaving the valve still incompetent. Such a flea is presumably more dangerous than the one whose proventriculus is completely blocked, since the contents of the stomach can be regurgitated into the wound with greater freedom, as long as the infection persists.

Further experiments were done on blocked fleas by Eskey (1938) and Eskey and Hass (1940). Their experiments led to the study of vector efficiency as some fleas, particularly *X. cheopis*, were found to be more efficient vectors than others. The average capacity of a rat flea stomach is $\frac{1}{2}$ cm. The blood of plague infected rat may contain between 10,000 to 100 million *Y. pestis*. Thus on a full feed a few to 5,000 organisms would be imbibed. These bacilli multiply in the proventriculus often to such an extent as to block the passage completely and prevent access of the food into stomach. Such a flea is hungry and when a new host is found it attacks with vigour (Bacot and Martin, 1914). The ability of the flea to become infected depends on the number of plague bacilli in the blood stream of the sick and dying rodents. The larger the number of rats dying of plague, the larger is the number of infected fleas released from their bodies, and the greater is the possibility of transfer of infection to human beings. Vector efficiency is also an important factor. Actually, houses with multiple cases generally showed a stronger evidence of rat mortality than those where only single cases were found.

Vector Efficiency

The studies of the Indian Plague Commission indicated the existence of

difference in vector efficiency between the various flea species and this problem engaged the attention of many workers. The methods used were mainly (1) mass transmission tests and (2) individual feeding experiments.

A great advantage of mass experiment tests is that these are generally conducted under conditions resembling those in nature, but this can be used preliminary to individual feeding tests which alone can determine the vector efficiency of species. Accordingly, Wheeler and Douglas (1941) first carried out tests *en masse* with each species, by using laboratory reared fleas and mice and then individual feeding tests. The information obtained by well-controlled examination of the fleas and mice established (1) the infection potential based upon the percentage of the fleas of a given species in which *Y. pestis* had become established; (2) the vector-potential based upon the percentage of infected fleas capable of transmitting the plague; and (3) the transmission-potential *i.e.*, the mean number of transmissions effected by the infective fleas. The product of these three factors represented the number of transmissions effected by a given number of fleas or the vector efficiency. They noted that *X. cheopis* had the higher infection-potential than *D. montana* but only half as many cheopis became infective and transmitted only half as many times. From these data, it may be concluded that an accurate evaluation of vector-efficiency includes knowledge of infection-potential, the vector-potential and transmission-potential. Burroughs (1947) ascertained the vector-efficiency of various fleas and found the true vector-efficiency of *X. cheopis* as 0.66 ± 0.234, that of other fleas being much lower (0.0015-0.250). There was, however, difference of opinion between different workers as to the comparative efficiency of male and female species but Webster and Chitre (1930 a and b) concluded that with experiments carried out on a larger scale the difference between the vector efficiency of male and female species became merged. The experiments carried out by ectoparasites other than rat fleas yielded negative or very irregular results. It is thus now generally understood why rodent flea si ngeneral are more effective as carriers of plague than other blood sucking ectoparasites such as bed bugs, lice, ticks and mites *etc.* which can also ingest and excrete plague bacilli but seldom or never infect by direct bites. It is also clear that sharp distinction must be made between infected and infective fleas.

Relative Vector Efficiency of X. cheopis, X. astia and X. brasiliensis

Hirst (1913, 1933) found *X. astia* to be a far less efficient plague vector than *X. cheopis* by his exhaustive investigations. The findings were confirmed experimentally through laboratory investigations, by Taylor and Chitre (1923), Goyle (1928), Webster and Chitre (1930 a and b), as well as through field surveys conducted by Gragg (1923) and King and Pandit (1931). A general agreement thus reached that although *X. astia* is capable of becoming blocked and of transmitting plague it is markedly less efficient vector than *X. cheopis* or *X. brasiliensis*. *X. brasiliensis* is, however, as efficient as *X. cheopis*, if not more. If less efficient vector is present along with the

efficient vector, it might play a subsidiary role only.

In South Africa, *X. philoxera* and *X. brasiliensis* proved to be equally efficient vector (Davis, 1939). Hopkins (1949) considered *X. brasiliensis* as the normal initiator of plague in Uganda and Vincke and Devignat (1946) found *X. cheopis* and *X. brasiliensis* to the predominant vectors in Belgian Congo, while Roberts (1935, 1936) stated that *X. brasiliensis* was the principal vector in rural and *X. cheopis* in urban Kenya. In India, *X. cheopis*, was the commonest vector except in the Western Ghats where *X. brasiliensis* was found to be the vector (Sharif and Narasimhan, 1945). In Brazil, *X. brasiliensis* was the common vector and in Hawaii Islands it was *X. vexabilis hawaiiansis* (Eskey, 1934).

Duration of Infectivity of Fleas

The length of time that a flea can remain infected is of great importance in regard to the transfer of infection in an epidemic from place to place. In 1950-51, this question had gained special importance in connection with the allegation by the Peruvian Government that the infected fleas have been tranferred from Calcutta to Peru though jute bags. The period of infectivity, however, depends on several factors, chief of which are humidity and temperature. During the epidemic the rat fleas may remain infective for 15 days, but during the non-epidemic season this period is reduced to 7 days (this is an experimental observation). Blocked fleas may live for 50 days in moist air at temperatures ranging from 50° to 59°F and for 25 days at 80.6° F. Fleas of wild rodents even after 150 days of starvation at 32°-59° F were able to transmit infection to rodents (Golov and Ioff, 1926). A fasting flea may remain alive for 47 days (in one instance, it lived for 96 days). For survival of the bacilli in the flea a low temperature of about 50° F and high humidity are favourable; 80°F or lower temperature with dry atmosphere is adverse to survival.

Types of Fleas Transmitting Plague and their Bionomics

The important transmitter so far known in the tropics is *X. cheopis*. That there is a correlation between the plague season and *X. cheopis* season is shown in Fig. 22. The other two types of fleas *viz.*, *X. astia* and *X. brasiliensis* found on rats are very poor transmitters. In Uganda, however, *brasiliensis* is a more important vector of plague than *X. cheopis*. In the temperate climate *Ceratophyllus fasciatus* and sometimes *Ctenophthalmus agyrets*, *Ctenocephaus canis* and *felis* transmit the infection. *Leptopsylla musculi* (mouse flea) is not important as vector. *Pulex irritans* from plague patients were found to infect guineapigs and survived for about 2 months.

Habits of the fleas are sometimes responsible for the difference in transmission and geographical distribution. For instance, *C. fasciatus* which has to have blood of half digested blood of its host in the larval stage, less frequently rides on the host than *X. cheopis* and hence the latter is more dangerous than the former. Their larvae feed on rice in stores where the hosts

frequent. In fact, the larvae of both *X. cheopis* and *X. brasiliensis* can readily thrive in the burrows of domestic rats even when the nutritive value of the larval food is very low but *X. brasiliensis* being less tolerant of temperature is generally found in the cooler region. In contrast. *X. astia* which requires rich larval food, flourishes in the burrows of *Tatera indica* and *Bandicoota malabarica* and sometimes domestic rats.

The temperature and humidity of atmosphere affect the growth of larvae and pupae. 0°-2°C is fatal for them. Breeding starts above 55°F with humidity above 80 per cent. A mean temperature of 88°F or temperature above 95°F reduces the virulence and number of plague bacilli in the stomach of the fleas leading to fewer successful transmissions. High temperature also reduces flea breeding power. *Cheopis* breeds well at 70°-80°F with humidity above 60 per cent. In this respect the latter can stand a little higher temperature than *astia.*

Burroughs (1947), Eskay and Haas (1940) and McCoy (1911) made comparative studies between *X. cheopis* and *N. fasciatus* and came to the conclusion that although *N. fasciatus* was a fairly effective vector it played a far less important role in the transmission of plague under natural conditions than *X. cheopis,* the latter being effective even in the winter months of Japan and in colder regions of Europe. *N. fasciatus* (var *nilgiriensis*), the flea mainly infesting *B. bengalensis* kok in Nilgiri Hills and *N. londilensis* in highlands of Ecuador supposed to be participating in plague transmission along with *X. cheopis* were found to be only poor vectors (George and Timothy, 1941 and Macchiavello, 1948).

Among other rat fleas which were found to be capable of transmission of plague infection may be mentioned the following: *Leptopsyllus segnis* (Eskey and Hass, 1940) in China, *Stivallius cognatus* (Swellengrebel and Otten, 1914) in Java, *Synopsyllus fonquerniei* (Robic, 1937) in Madagascar.

Transmission experiments with *P. irritans* became successful only on using numerous infected fleas (minimum five). Wu Lien teh and Pollitzer (1929) succeeded in transmitting infection into guineapigs with groups of two and three *P. irritans* respectively which had been collected during the plague outbreak in Tung-liao area of South Manchuria in 1928. But Jethmar (1930) working in the same village next year, when the plague situation was less severe found no further evidence of incriminating *P. irritans.* Girard (1943) however, maintained that *P. irritans* played an important role in certain plague areas in Morocco in the spread of plague infection from man to man. The importance of this flea and of the human louse was particularly emphasised by Blare and Baltazard (1945) in respect of interhuman transmission although admitting the role of rodent flea as the initiator of all plague outbreaks. *P. irritans* was also suggested as the possible transmitter of infection in European plague. However, the role of this flea was found negligible in China and Madagascar.

Role of Fleas in the Perpetuation of Plague

Many experiments were conducted to investigate if any role is played by the fleas in the perpetuation of plague infection. Otten (1932) noted that the infected flea *X. cheopis* under suitable climatic conditions could convey plague infection up to 43 days, *N. fasciatus* could do so up to 47 days (Bacot, 1915). According to the experiments carried out by George and Webster (1934) starving fleas were capable of transmitting plague up to 21 days. With occasional blood meal as it happens in the burrows of wild rats the length of life would be prolonged and infection maintained longer. Girard (1943) and Robic (1937) by exhaustive studies demonstrated the importance of free living *X. cheopis* in the perpetuation of plague.

(i) *Role of Wild Rodent Fleas*

In an interesting experimental investigation in South East Russia, Golov and Ioff (1926) kept three kinds of fleas—*Ct. resquorum, N. setosa* and *Ct. breviatus* at temperature corresponding to those of rodent burrows and found the infected fleas to survive up to 206 days, the plague bacilli in their faeces and stomach remaining virulent. The fleas survived at temperature 14°-27°C for 220 days and at 0°-15°C for 396 days. It was therefore, concluded that the fleas could play the important role of perpetuation of plague. In South Africa, Pirie (1927) proved that non-hibernating wild rodent fleas were capable of playing the analogous dual role by carrying over plague infection for 2-3 months or more.

Tumanosky and Poliak (1927) collected fleas from rodents five months after an epizootic and found some of the *N. setosa* and *Ctenophthalmos pollex* infected with plague. Similar experience was narrated by Barrera (1939, 1940) in Mendoza, Argentina. Prince and Wayson (1947) in Western USA placed infected *D. montana* fleas on hibernating squirrels, *Citellus townsendi*, and recovered them after 4 months, at least 4 of them had retained plague infection. These observations support the hypothesis that plague may be carried over the hibernating period not only by the rodents but also by their vector fleas. A cautious note was, however, issued by Eskey and Haas (1940) and Meyer (1942) in accepting the role of wild rodent fleas as stated above.

(ii) *Flea-borne Plague through Travellers and Transport*

The Indian Plague Research Commission (1907) with the aid of a group of 26 guineapigs collected 2 rat fleas and one human flea from the beddings and clothings from a house where plague cases had occurred. Only one of these guineapigs contacted plague. While it was generally agreed that plague infected fleas might be carried on the persons or in the baggage of travellers, opinions vary considerably as to its frequency and actual epidemiological importance. Flu (1921) and Pollitzer (1954) stated that transport of rice played the principal role in dissemination of plague in Central and South China. Similar experience was quoted by Sorel (1937) in Madagascar and Petrie *et al* (1923) and Hirst (1931) in Ceylon in the transfer of infection

from Rangoon. Long and Mostajo (1934) stated that Peru received infection from Calcutta through jute bags. However, it is true that infected flea may also serve as agents in the transfer of plague by the above process if some instances, although Pollitzer (1954) expressed doubt about the long-distance transfer of infected fleas in jute bags, such as from Calcutta to Peru.

(iii) *Subterranean Epizootic*

In some rural areas, the high external temperature prevents *X. cheopis* from living outside the rat nests where the temperature is 9°-14° cooler than outside. Under these circumstances plague infection is found in the nests where the fleas remain feeding on the young rats. A subterranean epizootic follows, slow in progress and with very little human involvement. Should the weather changes with drop in temperature, as happens during the rains, the fleas may leave their shelter, and the epizootic then comes to the surface. This explains the abrupt onset of plague which often follows the rains.

(iv) *Fleas as Carrier of Infection*

It is now believed that during the inter-epidemic period (*i.e.*, between the seasonal outbreaks) the infection is maintained in the fleas rather than in the rat population. According to some workers (George, 1935-37; Webster, 1931; Wassilieff, 1933) it is the infected fleas which carry over infection from epizootic to epizootic or smouldering epizootic occurs all the year round. The rat nests remain inhabited by infected fleas after a large part, the rat population had died. The remaining rats are immune to plague and the disease would soon die out (the virulence of plague also decrease in fleas) but susceptibles arrive either through or by migration and the infection is carried on. It is thought that decreasing virulence of plague in fleas may be responsible for mildness of human cases at the end of epidemics. Infective fleas were found in the burrows of Susliks 5 months after an epizootic among them in April-June (Pollitzer, 1954). However, the observations do not provide satisfactory explanation of the reappearance of plague in Calcutta after its virtual absence from this city for about a quarter of a century.

Summary of the Modes of Transmission

The reservoir of infection is mainly heterologous and rarely homologus. Homologous (man to man) transmission takes place through droplets as in pneumonic plague, through human flea, *Pulex irritans* (rare), or accidentally during autopsy or laboratory work.

Whereas heterologous transmission requires rodent—flea—man passage. The main species of rodents serving as reservoirs of plague infection in India are *R. rattus*, *R. norvegicus*, *Nesokia bengalensis*, *Gunomys varius*, *Mus musculus*, *Bandicoota indica* and *Crocidula cerulea* and occasionally wild rodents such as, *Mellardia meltada* (soft footed field rat), *Gunomys kok* (mole rat), *Leggarda booduga* (Indian field mouse), *Leggarda platythrix* and

Funumbulus pennati (tree squirrels). The reservior of infection is gerbil in South Africa, spring hare in Africa, squirrel, Chipmunk and marmot in N. America, spermaphiles and susliks in Russia and tarabagan in Manchuria. The transmission is normally heterologous following an epizootic or enzootic especially among *R. rattus* (house rat) in India. The interepidemic period is generally bridged over by setting up a chronic infection among rodents or by maintaining a chronic carrier condition among them (Seal, 1961), the wild rodents often playing an important role as chronic reservoir particularly in Central and Northern Asia, Russia, North America and Africa, by interchanging fleas with their domestic species and through migration. For instance, tarabagan and mormot harbour chronic plague infection which remains latent during hibernation period (without developing resistance against the organisation), and is spread during the dry season when they go in out search of food and are eventually handled by men. Some of these rodents and animals including mice migrate in search of food during harvesting and transmit infection to domestic rats.

Human bubonic plague cases not complicated by the involvement of respiratory tract cannot transmit directly. Even if the buboes burst, the pus contains a few, if any, virulent plague bacilli. Cases of man to man transmission, though rare, have been suggested through human flea—*Pulex irritans*. Thus the infection is transmitted to human beings almost exclusively by rodent, fleas. The relationship of rodent, flea and man in the transmission of plague infection is given in Fig. 24.

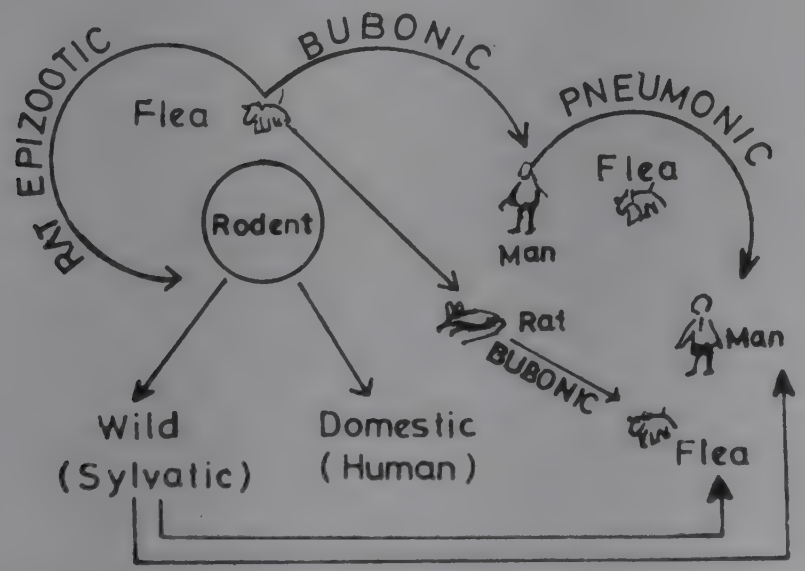

Fig. 24. Plague epidemic cycle—Relationship of rodent, flea and man in the transmission of plague infection.

Phase V of Evolution of Knowledge on Plague—Newer Developments

Phase V is the last phase in the development of knowledge about plague, which began from the mid-twentieth century and is characterised by the newer development of knowledge about the plague bacillus and allied organisms. During this phase significant advances were made in the knowledge of plague by means of experimental studies on plague transmission, microbiology of plague bacillus, immunological studies of plague bacillus and allied organisms, serological methods for the diagnosis of plague, methods of differentiation of *Y. pestis* and *Y. pseudotuberculosis*, diagnosis and treatment, and control and prevention of plague. These advances have been discussed in separate chapters in the following pages.

CHAPTER VI

Experimental Studies on Plague Transmission

The Role of Movement of Rats in the Spread of Plague Infection

A divergence of opinion existed on the spread of plague through direct and long range migration of rats. There were many who supported the view that an exodus of rats takes place at the onset of plague epizootic to near and distant places (Wilkinson, 1904; Bannerman, 1906; Eltion, 1925; Mitchell, 1927; Evans *et al*, 1943; and Macchiavello, 1948). The other school of thought doubted the above mode of local spread and observed only short range (not exceeding 200 yards) movement of rats. In its opinion the spread of plague *per saltum* was affected by passive transfer of rats and fleas through human or goods traffic or by contact with the adjoining rat colonies (Hankin, 1905; Indian Plague Research Commission, 1907; Petrie *et al*, 1923; Hirst, 1926-1927; Venables and Leslie, 1942; Meyer, 1942; Davis *et al*, 1948; and Baltazard and Bhatnagar, 1958). The author's personal experience of the 1948-49 epidemic in Calcutta led to the strong suspicion of metastatic spread of infection by creation of distant foci through migration of infected rats, as the dead rats collected from distant parts of the city within a few days after the first human case proved bacteriologically positive (Lal and Seal, 1948). For instance, infected dead rats were almost simultaneously found in Wards 1, 23, 25 and 31 far away from the original focus in Ward 8 (Fig. 25). It was, therefore, thought that the dispersal of plague infection in the earlier part of the epidemic (epizootic) might have been due to direct rat movement. An investigation was, therefore, undertaken by Seal and Bhattacharjee (1961) to study the range of movement of rats in Calcutta, both for long and short distances, in the hope that the distribution of plague cases in the city could be adequately explained.

Long Range Movement

Rats were captured by Wander traps from different wards of the city (32 in all) and brought to the Institute Laboratory under canvass cover. They were freed from the fleas and kept under observation for varying periods to avoid incubating infection. They were then tattoo-marked in their ears with

Fig. 25. Map of Calcutta and Howrah showing incidence of plague cases and deaths during 1948 and 1949.

different symbols to different batches for identification, carried in a van in covered cages to some distant outskirts or to another new ward of the city and released. The distance between the original place of capture and spots of release were noted. Efforts were then made to recapture them in the city, particularly from the places of their original capture and release and also at intermediate stations (Fig. 26).

During the period of study a total of 1905 rats (854 male and 1051 female) marked in 20 batches comprising 148 *R. rattus*, 161 *R. norvegicus*, 1544 *B. bengalensis*, and 52 *B. indica* were released. Ultimately 7 of them were recaptured (1 *R. rattus*, and 6 *B. bengalensis*), the interval between release and recapture being 9 to 264 days. Except one which travelled 1.5 km, the others travelled between 4.5 to 6 km, the majority being re-captured

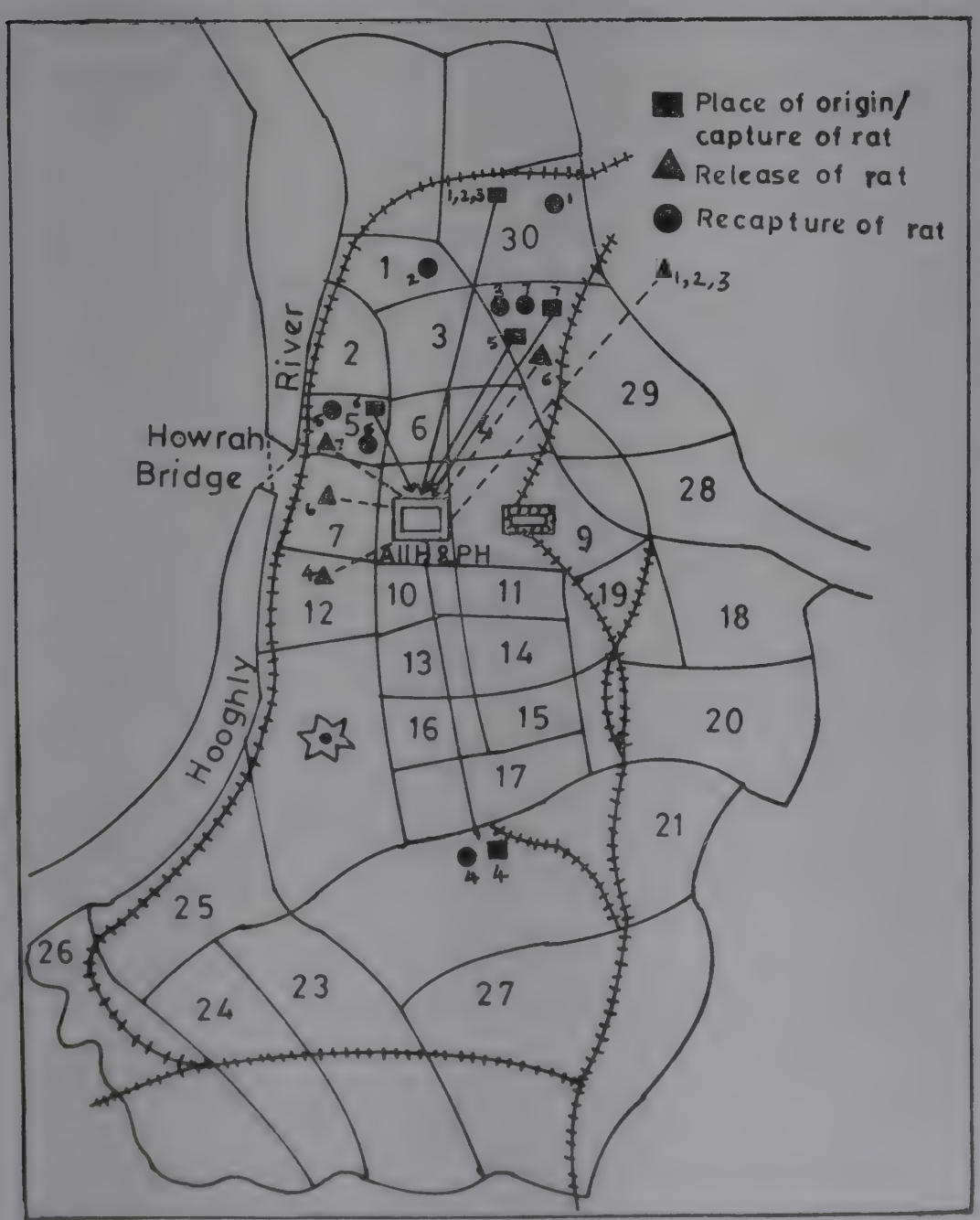

Fig. 26. Map showing long range migration of rats in Calcutta in 1949.

within 2-3 weeks either near the original place of capture or from intermediary places. The total excursion from the place of capture to the place of recapture varied from 9 to 24 km.

Short Range or Daily Excursion

To find out the daily range of excursions baits of wheat dough 3/8″ diameter containnig a few grains of methylene blue or eosin red were prepared and 50 such baits were placed every evening near the mouths of rat burrows in three types of areas *e.g.*, (i) purely residential; (ii) mixed residential and shop area; and (iii) purely godown and factory area. Dyes were excreted in the droppings within 24 to 48 h (as noted by laboratory experiment). Search was regularly made for these coloured droppings for at least 4 or 5 days after placing them, in the premises, neighbourhood and within a radius of 200 m. The colouring was confirmed in the laboratory. Traps were also laid systematically in and around the area within 200 yards of baiting and the rats thus trapped were identified and then watched for the passage of the coloured droppings or urine, if any. Some of them were also killed and post-mortem done to detect the dye. The period of observation was six months.

Rat droppings could be collected in about 30 per cent of daily placements and the total number of rats trapped was 467. The types of prevalent rats were *R. rattus*, *R. norvegicus* and *B. bengalensis*. Colours were recovered both from the rat droppings as well as from the trapped rats. By this method it was possible to trace out the paths and range of rat movement. The distance travelled was generally up to 50 yards, but there was considerable intermingling of groups forming colonies.

Distribution of Plague Cases vis-a-vis Proximity of Rat Falls

From the results of analysis it was found that the risk of contracting plague was greatest where infected rats were detected within 50 yards (45 m) and thereafter it progressively declined, particularly if the distance was more than 180 m. This might help in deciding about the area of cordoning and production of barrier by spraying DDT and adopting cyanogassing measures.

The above experiment showed that in the natural course of events rats move within 45 m and perhaps not beyond 180 m. Such findings were also reported by Spencer and David (1950) and Kartman and Lonergan (1955) from Hawaii Islands.

In respect of long range movement these animals have a natural tendency to come back to their group in spite of the risk of retracing their paths extending over several kilometers. Now for the experimental evidence to show that they intuitively run away from the initial epizootic area marked rats were released in the same area in the hope that with the reappearance of the next episode of epizootic the range of movement could be ascertained by the markings but as no epizootic followed during the next season (due perhaps to the intensive antiplague measures taken) the observation made

at the onset of the epizootic could not be confirmed by actual experimental observation. However, *prima facie* the collection of dead rats positive of plague infection simultaneously from distant wards of the city within a day or shortly after the first positive human case would not have been possible without the exodus of these rats from the original spot of the epizootic (Lal and Seal, 1948).

The Role of Domestic and Peridomestic Rodents in the Maintenance of Plague Infection and Variation in Virulence of the Organism

No completely satisfactory explanation had been found for the periodic rise of plague in endemic areas. For the seasonal recrudescence the influence of the breeding habits of rats and fleas have been fairly worked out, but the Russian (Nikanaroff, 1925; Tinkar and Kalabuchov, 1934; Lebanov and Fedorov, 1938) and American workers (Harrison, 1920; Meyer, 1942) thought that it was associated with the seasonal variation of susceptibility of the prevailing rodents to the infection. Pollitzer (1954), however, suggested that the link was maintained during off-season by the rodents themselves suffering from acute or subacute plague with bacteraemia. According to him, even if this mechanism failed, fleas by harbouring *Y. pestis* were apt to carry over the infection. According to Meyer and Eddie (1938) the sylvatic plague probably persists in the area once invaded. The question thus arises where and how the infection is actually maintained. Both rats and fleas have been suggested by different workers to harbour the infection but the maximum period for which they have been found to carry the infection by the available methods does not explain the recrudescence of plague at long intervals of time.

Wild rodents often acting as natural reservoirs of plague infection have been found widely distributed in the world as listed by WHO Expert Committee in 1959. A fairly extensive work on the role of wild rodents has been conducted in USA by Kartman *et al* (1956, 1958). In India, such natural infection in wild rodents had been reported in stray instances by Simond (1898), Hossack (1906), Indian Plague Commission (1907, 1910), George and Webster (1943), Sharif and Narasimham (1941) and George and Timothy (1941), the rodents involved being *Tatera indica*, *Mus boodooga*, *Mellardia meltads*, *Bandicoota indica* and *Malabarica*, *Gunomys kok* and *B. bengalensis.* However, except the first three, they were all peridomestic rodents mixing freely with the domestic ones. Rao (1947) on the other hand, found in *Tatera indica* fully susceptible and *R. rattus* resistant to infection, thus excluding wild rodents as possibe reservoirs.

On the whole, the Indian Plague workers did not find any convincing evidence of plague infection being maintained in wild rodents. Lately, however, Baltazard *et al* (1958) found infection in *T. indica* in certain parts of Uttar Pradesh and propounded the hypothesis that instead of a permanent focus, the infection moved continuously by contiguity from place to place through the movement of these rats.

Among the possible roles of wild rodents and fleas in maintaining plague infection during the inter-epidemic period, the possibility of low grade infection remaining in the chronic foci or in dormant state in the commensal rats was also supported by a number of workers (Simond, 1898; Ledingham, 1907; Swellengrebel and Otten, 1914; Bordas *et al*, 1922; William and Kremerer, 1923; and Lal and Seal, 1949). Among the Indian workers, George (1935-37) working in the Cumbum Valley in South India found infection among commensal rats during the off-season by means of animal inoculation test, while similar tests done on thousands of wild rodents yielded only negative results. Lal and Seal (1949) working in Calcutta during 1948-49 outbreak found evidence of dormant infection among the peridomestic rodents, *B. bengalensis* and Chowdhury could isolate such infection as late as in 1957 (personal communication). Similar phenomenon was also observed among the surviving rats after the completion of immunity test with virulent plague organism. It may, however, be mentioned that such infection being usually of low grade virulence was likely to be missed by the usual bacteriologically tests but for an improved serological technique evolved by Seal (1951 a).

The Indian Council of Medical Research, after reviewing the position, felt the necessity of further research on the issue. Sudden appearance of plague in Assam in 1956-57 (Seal and Bose, 1957) further substantiated such a need. The investigation undertaken by the authors related to the persistence of infection in domestic and peridomestic rats and their vector fleas, change in virulence of plague organisms and that of immunity in rats in different seasons.

Experimental Devices

The experiments were devised on the principles of experimental epidemiology originally planned by Greenwood *et al* (1936). The devices set up for transmission experiment in the ordinary laboratory room consisted of two parts (a) glass chamber and (b) experimental cage enclosed in a glass box (Figs 27, 28).

The experimental boxes were made of glass plates with lids and bottoms made of tin sheets. The lid had four circular openings, two large ones to introduce two wiremesh cages for holding the rats and two small ones guarded by fine copper wiremesh to prevent the escape of fleas and for ventilation. Second guards, of thick wiremesh for preventing escape of rat by breaking the copper wiremesh were also fixed. Many such chambers were set up for simultaneous multiple experiments.

General Procedure

The general procedure was standardised by a set of preliminary experiments with both susceptible and immunised groups of rats. The former group studied the behaviour of epidemic with a continuous supply of susceptible rats and the latter group ascertained whether chronic or sub-clinical plague

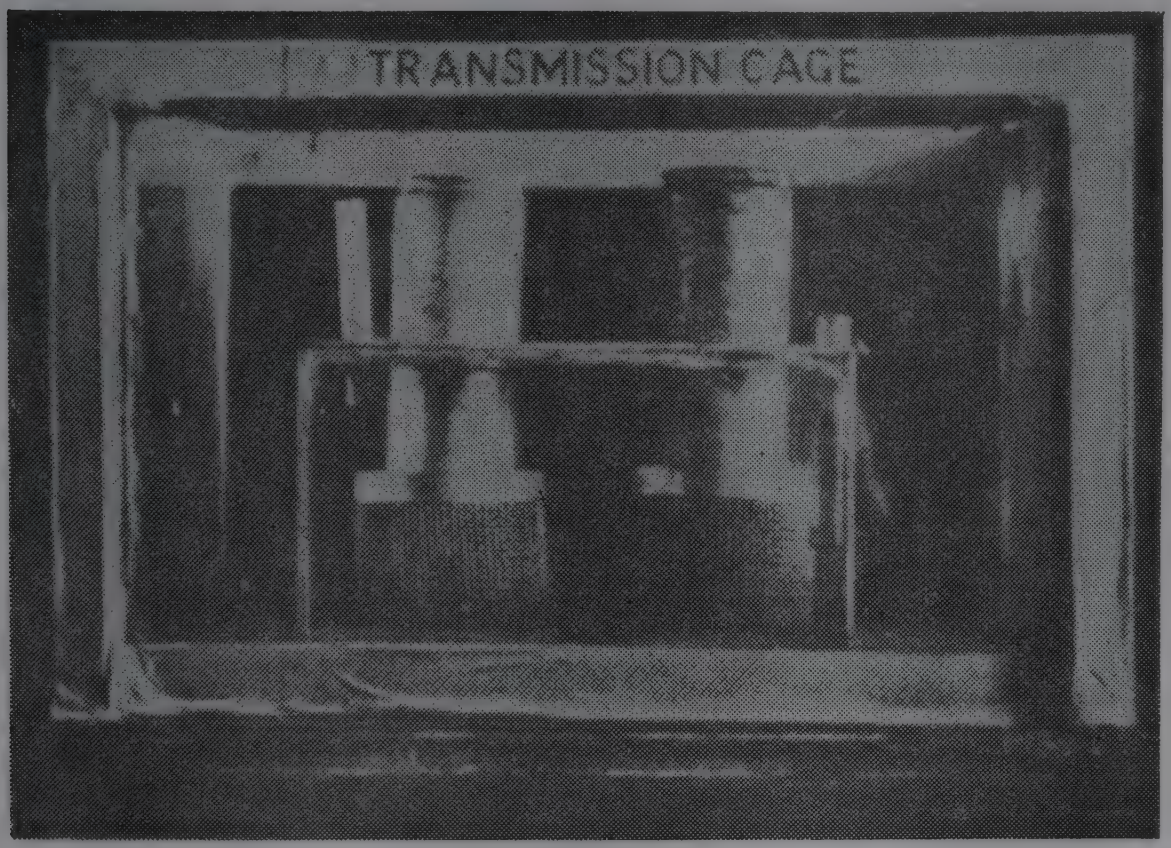

Fig. 27. Experimental transmission chamber with a glass box containing two wire cages.

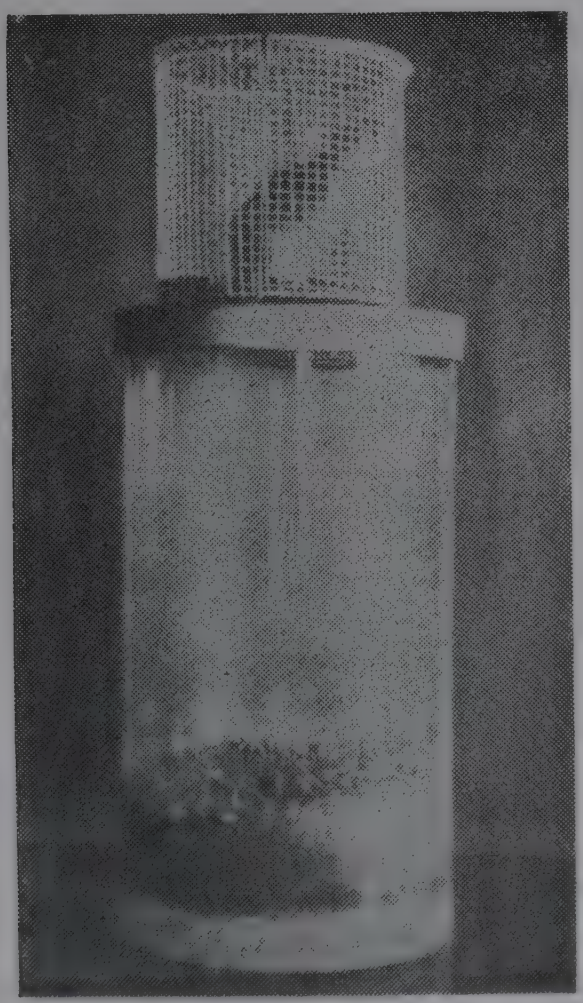

Fig. 28. A glass jar used for breeding of fleas with a live rat to feed the breed.

foci would arise by infecting them with virulent plague organism through fleas and to see if these animals would naturally infect the susceptibles and were capable of transmitting infection at any stage.

Susceptible commensal rodents were obtained from the field and after removing all fleas and necessary quarantine (10 to 15 days) they were subjected to plague infection through fleas bred in the laboratory. Natural transmission from rat to rat was continuously observed and post-mortem examination, isolation of organism and testing their virulence at different stages was done. Suitable vectors for the experiment were selected by the method of Wheeler and Douglas (1941).

For continuous transmission experiments in white mice and in immunised and susceptible rats fresh fleas were supplemented after death of each animal till it was noted that the fleas were actually breeding inside the experimental box.

Testing the Virulence of the Organism

The virulence of the organism isolated was tested first by Sokhey's (1939 a) method but when virulence was greatly reduced modified Jawetz and Meyer's (1944) technique was employed. The infectivity of such organism of reduced virulence was further tested by introducing fresh fleas and healthy animals into the experimental cage which yielded that organism. All experiments were carried out at ordinary room temperature varying from 12° to 35°C in different seasons. A large pure-bred flea population was maintained throughout the experimental period. 100 per cent susceptible rats were supplied by the Plague Control Laboratory of the Government of West Bengal, the susceptibility being tested in a sample from each batch. All experimental rats were fed with standard nutritive diet containing milk, halibut liver oil, bread, sprouted gram and occasionally carrot.

Immunisation of Rat

Certain number of fully susceptible *R. rattus* and *B. bengalensis* collected from the plague-free Ward 10 were subjected to partial immunisation by using half the doses of either Haffkine Plague vaccine or specific plague protein antigen A of Seal (1951) for transmission experiment with a view to watch its effect on the maintenance of inapparent or chronic focus of infection in them. The actual doses used were 0.02 ml vaccine and 0.05 mg of the protein antigen divided into two equal doses and injected subcutaneously at intervals of 1 week and the animals were used for experiment not earlier than 1 week after the last dose.

Summary of Results of Experimental Procedures

The methodology adopted for the experiments was continued for a period of four year and 10 series of experiments were carried out within this period (Seal and Bhattacharjee, 1961). The study was undertaken to elucidate the role of domestic *R. rattus* and peridomestic *B. bengalensis* in the mainte-

nance of plague infection during the short and long interepidemic intervals in Calcutta. Both non-immunised and immunised rats were used in various combinations as were likely to be found in nature. Immunisation was done by giving half the normal dose of Haffkine plague vaccine and specific plague antigen in two equal divided doses to separate groups for creating partial immunity. *X. cheopis* which proved more efficient vector than *X. astia* was used for the transmission experiment. The average normal cage life for *R. rattus* and *B. bengalensis* was 86 $\pm$ 11 and 73 $\pm$ 10 days respectively.

In an experiment with non-immunised *R. rattus* extending for nearly a year fluctuation was noted in the incubation period in different seasons, generally rising during the second half of the year (average temperature about 80°F) and falling during the latter part of the winter and earlier part of spring (average temperature varying between 68° and 78°F). This behaviour roughly corresponded with the natural seasonal rise of plague in Calcutta. Apart from this change serial transmission experiments led to the reduction of virulence of the organism and permitted the rats to harbour the infection in an inapparent or subclinical form for prolonged periods *e.g.*, 114 days in non-immunised rats and 101 days in immunised rats. If these rats were not killed for isolation of plague bacilli in all probability their lives would have much prolonged thus serving as a carrier of infection for a still longer period.

Experiments with partially immunised rats showed that the longevity was generally increased but the majority of them ultimately succumbed to the infection. Some of them, however, remained alive for a long period and on killing they were generally *Y. pestis* negative; only in a few instances they were *Y. pestis* positive with very little or no pathological evidence. The organism isolated in such cases was of low virulence, the m.l.d. ranging between 119 and 157 against an m.l.d. of 15 to 30 organisms of the original virulent strain. They were, however, capable of regaining their virulence causing quick death of the susceptible rats when exposed to them. In one instance the virulence was so much reduced that even 1 million organisms could not kill mice and it was artificially regained by three serial mouse passages.

It was further observed that two doses of immunisation with specific plague protein antigen A produced better immunity than the killed Haffkine plague vaccine and *R. rattus* was found more vulnerable and responsive to plague infection through fleas than *B. bengalensis*. Also, comparatively large number of non-immunised rats which survived for long periods were carrying *Y. pestis* in subclinical state than the immunised ones.

The fleas in these experiments obtained infection when the hosts had bacteraemia just prior to their succumbing to the infection and once the infection was imbibed by them it was retained up to 2 weeks or more to transmit the infection to freshly introduced rats even in the absence of infection in the immediately dying rats. However, failure to isolate organisms did not always mean absence of infection. In an experimental epidemiological

study with mouse typhoid organism Topley (1928) came to the conclusion that 'Strain of low virulence may persist for long periods in the tissues of the originally infected hosts . . .'. This therefore is a common phenomenon in the host-parasite relationship of human and animal diseases in the perpetuation of infection and in the rise and fall of epidemics. Altogether 15 experiments were carried out, the last two being undertaken to catch the bacteraemic condition in the partially immunised groups of rats. These experiments provided some evidence in favour of the view that in a city, such as Calcutta, the infection of plague was maintained in local rat population, particularly in the partially immunised rats in sub-clinical or inapparent form for prolonged periods and then either they themselves relapsed into clinical form with bacteraemia and death or into sub-clinical form with bacteraemia followed by recovery and rise of immunity.

Two experiments were carried out to detect the supposed bacteraemia and the number of organisms per cm square on slide or number of colonies on blood agar medium per cm. But except at the time when the animal was actually sick or only 24 hours earlier that a positive blood culture could be obtained. However, a subsequent observation by Deoras (1960) from Bombay lent support to this view. Specimens of *B. bengalensis* collected from the congested area of Kumtipura which were previously fully susceptible acquired 45 per cent resistance against plague infection since last tested, without however, any evidence of frank epizootic or human epidemic in the area. Evidently, chronic or sub-clinical type of infection must have brought about this change.

Thus in the perpetuation of infection organisms of low grade virulence seemed to play more important role than the virulent organism; and if suitable environment was available and sufficient susceptibles accumulated to facilitate circulation of the organism, the case incidence among rats assumed epizootic proportions with or without involving human beings.

Rodent Distribution, Density and Other Factors

The other important factors like the natural distribution of rodents in the area, their densities, growth characteristics and susceptibility or resistance to plague infection (herd structure), higher proportion of *R. rattus* and *R. norvegicus* was found favourable for dissemination of plague. The seasonal incidence of plague also seemed to be associated with the bionomics and growth characteristics of rat population in plague transmission. According to Habbu (1960) rats acquired varying degrees of resistance following epidemics of plague. It was thus worthwhile to include some of these factors in the investigation.

Rodent Distribution and Densities

The relative distribution of species of rats in Wards 8 and 10 in different seasons is given in Table 19.

Table 19. Rodent densities in wards 8 and 10 by species and season (1952-53)

Season	Rodent densities in Ward 8 (endemic)				Rodent densities in Ward 10 (non-endemic)			
	Total	R.r	R.n	B.b	Total	R.r	R.n	B.b
Jan-March	22.6	7.1	1.4	13.3	23.2	8.6	0.7	13.9
April-June	42.5	18.1	6.1	10.2	10.2	0.0	3.4	6.8
July-Sept.	21.8	4.1	5.3	10.9	10.9	2.7	2.1	6.1
Oct.-Dec.	30.7	5.4	2.7	32.1	12.1	2.6	2.3	27.2
Whole year	29.8	8.5	3.9	22.5	12.5	3.8	1.9	16.8
Rats caught	498	148	65	291	445	75	38	332

R.r= *R. rattus*; R.n= *R. norvegicus;* B.b= *B. bengalensis.*

Resistance Test of Rodents from Wards 8 and 10 (Calcutta City)

One hundred and forty four rats from Wards 8 and 10 were subjected to resistance test by injecting a fixed dose of 5000 virulent *Y. pestis* as standardised by the Haffkine Institute, Bombay. The results are given in Table 20.

The results show that all specimens of rats in Ward 10 (plague free) were fully susceptible whereas at least 14 per cent of rats in Ward 8 (plague infected) were resistant (contributed mostly by *R. rattus* - 40%).

Further Studies on the Ecology of Rodent Fleas

Seal and Bhattacharjee (1961) carried out experimental studies on the bionomics of *X. cheopis* and *X. astia* in relation to the epidemiology of plague in Calcutta (1948-1952). One of the impressions that gained ground during the investigation of this epidemic was that the infection was indigenous and not imported. It was also observed that the occurrence of human cases of plague was not associated with high *cheopis* index except for a short period in certain localised areas. Even the *astia* and total flea indices were much below those observed by Rao (1936) when the city was free from human plague cases. The review of literature (Seal and Bhattacharjee, 1961) revealed contradictory findings. It was observed that in a ward of the city where plague had been occurring every year between 1948 and 1951 the *cheopis* index was higher during the plague season than in the adjacent wards in which only stray cases of suspected plague were reported in 1949. Thus a need was felt to study the bionomics of fleas in India by simultaneous and coordinated field and laboratory studies, with a view to determine the seasonal, environmental and other conditions permitting or otherwise influencing the breeding of insects at various stages of their life cycle and their relationship, if any, with the prevalence of plague in the city and the endemicity of plague in general.

The life cycle of *X. cheopis* and *X. astia*, the two prevalent species of rat fleas in the city was followed from egg laying to emergence of adult fleas in

Table 20. Resistance test of rats caught in Wards 8 and 10 (1953-54)

Type of rat	Ward 8		Ward 10	
	No. tested	No. died	No. tested	No. died
R. rattus	30	18 (60.0)	22	22 (100.0)
R. norvegicus	28	26 (92.8)	18	18 (100.0)
B. bengalensis	50	49 (98.0)	55	55 (100.0)
Total	108	93 (86.0)	95	95 (100.0)

Figures in parentheses represent the precentage mortality.

the laboratory at the prevailing room temperature and humidity at all seasons of the year for which pureline breeding colonies of insects were built up in 1952 by modified Sharif's technique (Sharif, 1948) and maintained for more than 6 years in a series of glass jars. The temperature of the room was not allowed to go beyond 95°F at any time by means of *khus khus*. Rat droppings were added instead of powdered blood for larval nutrition. The technique devised by the author for examination of fleas was to collect them in trays with water and then remove each one by forcep to place on slide and cover it with coverslip; under the microscope distinct picture could be seen for identification. A summary of results of this study are given here:

1. It was observed that though reproduction of *X. cheopis* goes on all the year round, it has two main waves of growth one in the winter immediately preceding the epidemic wave of plague, and the other during the rainy season, whereas *X. astia* has only one main wave of growth in the rainy season extending to autumn (Table 21).

2. It was also found that starved *X. cheopis*, wild or newly hatched, generally lived longer than the similarly placed *X. astia* and those bred in the laboratory lived longer than those collected from the field. The feeding of blood meal to the newly hatched *X. cheopis* and *X. astia* resulted in an increase in the average survival period of 6.7 and 5.3 days respectively, the longest period of survival being 21.0 and 11.0 days respectively (both females). Thus *X. cheopis* seemed to be less hardy than *X. astia* so far as the condition in Calcutta was concerned (Table 22).

3. During the periods of growth *X. cheopis* females were found to increase greatly in proportion than males but the condition was reversed in case of *X. astia* (Table 23). These findings could be fairly corroborated in the field.

4. The study of the biting properties of the species showed that *cheopis* is normally a poor feeder on man but the propensity increased during winter and spring both for human and rat blood.

5. The results obtained by previous workers under Indian or similar conditions showed that the average longevity of wild and starved fleas did not

Table 21. Growth efficiency of *X. cheopis* and *X. astia* at different stages of their life cycles and different seasons as observed between April 1952 and August 1953

Season (1952-53)	Species	No. of eggs observed	Larva hatched %	Eggs to cocoon %	Eggs to adult %	Average time taken in days to complete the cycle
Summer (May-June)	*X. cheopis*	241	24.5	10.4	5.8	25.7
	X. astia	244	44.7	31.1	16.4	22.8
Rainy (July-Sept.)	*X. cheopis*	150	40.0	32.6	28.0	24.5
	X. astia	110	43.7	35.3	33.7	23.8
Autumn (Oct.-Nov.)	*X. cheopis*	90	75.6	11.1	4.4	30.1
	X. astia	127	81.0	31.5	31.5	28.0
Winter (Dec.-Feb.)	*X. cheopis*	172	78.4	44.2	37.2	31.6
	X. astia	113	88.5	30.0	24.5	32.4
Spring (March-April)	*X. cheopis*	196	44.4	25.0	18.3	28.0
	X. astia	105	57.1	34.3	23.8	23.4
Rainy (July-Aug. 1953)	*X. cheopis*	128	22.2	17.0	16.3	26.3
	X. astia	271	40.2	32.1	30.2	24.2
Average April 1952 to August 1953	*X. cheopis*	977	45.1	23.6	18.6	28.4
	X. astia	979	54.6	32.2	26.0	26.4

Table 22. Average longevity in days of wild and laboratory bred starved newly hatched male and female cheopis and astia fleas in different seasons

(A) Longevity of X. cheopis in days

Condition of fleas	Sex	Jan-Mar.		April-June		July-Sept.		Oct.-Dec.		Year	
		No. tested	Av. longev.	No. tested	Av. longev.	No. tested	Av. longev.	No. tested	Av. longev.	No. tested	Av. longev.
Wild and starved	M	115	2.0	93	1.7	116	0.8	96	1.5	420	1.5
	F	126	3.2	85	2.4	115	1.7	108	2.5	434	2.5
Newly hatched and starved	M	40	3.0	59	3.2	98	2.4	54	2.0	251	3.2
	F	94	7.0	117	5.8	112	4.7	73	5.5	296	5.7
	Both	134	5.5	176	4.6	210	3.6	127	4.3	647	4.5
(B) Longevity of X. astia in days											
Wild and starved	M	110	1.7	93	1.0	104	1.7	107	1.1	414	1.3
	F	91	2.4	116	2.3	141	1.5	115	2.0	464	2.2
	Both	201	2.2	209	1.0	248	1.1	220	1.6	878	1.8
Newly hatched and starved	M	76	3.2	46	2.0	49	2.4	23	2.8	194	2.9
	F	20	5.5	65	4.9	154	3.9	78	4.9	317	4.9
	Both	96	4.4	111	4.0	203	3.2	101	3.8	511	3.7

Maximum longevity in days

Type of flea	Wild and starved			Newly hatched and starved		
	M	F	Both	M	F	Both
X. cheopis	4½	6	6	9	14	14
X. astia	3	4½	4½	9	13	13

Table 23. Percentage sex distribution of laboratory bred fleas in 1952-55 as compared with wild catch in the city in 1949

Month	*X. cheopis*				*X. astia*			
	Laboratory bred fleas 1952-53		Wild catches 1949		Laboratory bred fleas 1952-53		Wild catches 1949	
	M	F	M	F	M	F	M	F
January	28.5	71.5	44.6	55.4	76.9	23.1	41.4	59.5
February	28.3	71.7	38.6	61.4	78.2	21.8	38.9	61.1
March	27.7	72.1	46.1	53.9	85.2	14.8	38.4	61.6
April	31.2	68.8	38.7	61.3	46.4	53.6	35.0	65.0
May	33.0	67.0	59.3	40.7	43.5	56.5	56.2	43.7
June	36.7	63.3	55.6	44.4	37.7	62.3	52.0	48.0
July	50.3	49.7	48.8	51.2	20.4	79.6	44.2	55.8
August	47.9	52.1	49.2	50.8	14.1	85.1	46.2	52.8
September	47.7	52.3	44.8	55.2	14.2	85.8	28.9	71.1
October	41.0	59.0	52.3	47.7	20.4	74.6	33.5	66.5
November	36.3	63.7	55.3	44.7	22.2	77.8	46.4	53.6
December	34.0	66.0	35.7	64.3	28.6	71.4	55.2	44.8
Total	38.3	61.7	46.7	53.3	33.7	66.3	36.3	63.7

Table 24. Average longevity of wild and starved fleas as noted by different workers

Author/s	Year	Place of study	Range of temperature, °F	Average range of humidity, %	Average longevity in days	
					X. cheopis	*X. astia*
Seal and Bhattacharjee	1961	Calcutta	56.4-100.5	45-88	2.1	1.8
Bacot	1912	Poona (now Pune)	54.4-84.0	56-98	2.6	—
Cragg and Swaminathan	1922	Agra	72.6-96.5	64-94	—	—
Hirst	1924	Colombo	80.0-83.1	77-83	2.83-3.81	2.93-4.16
Goyle	1928	Lucknow	58.1-79.6	52-84	2.3	2.2-3.6
Webster	1930	Bombay	81.6-86.0	78.6-90	1.74	1.98
Hopkins	1935	East Africa	59.0-86.0	80-100	—	5.3

Figures calculated from the published data.

Table 25. Average longevity of newly hatched laboratory bred fleas as noted by different workers at varying temperatures and humidities

Author/s	Year of study	Temperature range, °F	Humidity range, %	Average longevity days	
				X. cheopis	*X. astia*
Seal and Bhattacharjee	(1953)				
Burroughs	(1953)	77.9-94.7	78-96.2	4.15 (14)	3.9 (13)
		84.2	60.0	5 (8)	—
		68.0	56.0	7 (13)	—
		68.0	92.0	9 (26)	—
		55.5	65.0	4 (6)	—
		50.0	94.0	7 (34)	—
Bacot	(1912)	54.4-84.0	50.0-93	7.2 (25)	—
Webster	(1930)	68.0-98.4	0.3-0.405*	6.9 (28)	5.7 (17)
Leeson	(1932)	96.6	60.90	1.6 (3.6)	—
		80.0	60.90	4.5 (17.5)	7.5-12.8
		73.4	60	6.2 (14.5)	10.1
		73.4	90	—	11.7
Hopkins	(1935)	68.0	100	21.7 (36)	—

Figures calculated from the published data (*vide* list of references) and those in parentheses indicate the longest period of longevity.

*Saturation deficiency.

generally exceed 3 days (Table 24) while that of newly hatched laboratory bred fleas varied slightly under different conditions of temperature and humi-

dity (Tables 24 and 25). According to Bacot (1914) and Goyle (1928) cool and moist environment prolonged life while temperature higher than 90°F and relative humidity below 60 per cent shortened life as shown by Burroughs (1953). The longest duration of life of freshly hatched and starved fleas noted was 34 days for *cheopis* (Bacot, 1914; Webster, 1930; Burroughs 1953) and 17 days for *astia* by above workers and 14 and 13 days respectively by Seal and Bhattacharjee (1961).

CHAPTER VII

Microbiology of Plague Bacillus

After 1894—the year of discovery of the plague bacillus, till the middle of the present century attempts were made to obtain clearer and better ideas about the cultural, morphological, biochemical and antigenic characteristics of the organism and preparation of vaccine for preventive inoculation and of antiserum and chemo-therapeutic and antibiotic drugs for treatment of the disease.

General Characteristics of the Organism

The organism was initially classified under the genus *Pasteurella trevisan* along with *P. pseudotuberculosis*, *P. multicida*, *P. haemolytica*, and *P. tularensis* in the family of Parvobacteriacae in the Berghey's Manual of Determinative Bacteriology. Loghem (1946) proposed to class *P. pestis* and *P. pseudotuberculosis* under a new genus called '*Yersinia*' in honour of the discoverer of the bacillus. However, the organism continued to be classed as *Pasteurella* and not *Yersinia* till very late.

Culture Characteristics

For the study of the antigenic structure and isolation of the specific soluble substance of plague bacillus, needed for the preparation of effective vaccine and antiserum, the only available methods of prevention and treatment of plague prior to 1940, the nature and composition of the growth media play an essential role.

Growth on solid media: It appears that Kitasato (1894) used beef extract, Yersin gelatin medium and broth for cultivation of the organism. On cultivating the organism on agar slants a delicate, greyish white layer of growth was obtained which when touched with a platinum loop displayed a slimy consistency. This was considered by MacConkey (1908) to be a characteristic of the plague bacillus in contrast to the pseudotuberculosis bacillus.

Markl (1914) described two types of colonies on agar which corresponded to smooth and rough types as described by subsequent workers. However, ordinary agar slants were not considered by him suitable for growth of *Y. pestis*.

Kessel and Overbrook (1901) cultivated the plague bacillus on gelatin dishes at 20°C for 2-3 days with marked variation. An obvious drawback was that the contamination of material caused liquefaction of gelatin, thus interfering with the growth of *Y. pestis*.

Growth in liquid media: Liquid medium has been the commonest mode of cultivation of the organism. Haffkine (1897) used it for the preparation of vaccine. The growth started with floccules at the bottom and sides of the flask followed by the formation of a surface film with thread-like strands hanging down towards the bottom of the flask. This was further facilitated by adding a little oil (ghee) to the broth. The growth, known as 'stalactite growth', was supposed to be a characteristic of plague bacillus culture. The growth was apparently facilitated by oxygen hence, Haffkine deviseda flask, now known as Haffkine flask which provides a wider surface on the top of the broth for maximum utilisation of oxygen. Later observations indicated that this stalactite growth was of rough organism with much reduced immunogenic power. The author's work (Seal and Mukherji, 1950; Seal, 1951 a) on the media and on lag phase of the growth will be discussed under the new casein hydrolysate medium of Seal (1943).

Further Improvement of Growth Media

Sokhey and Habbu (1943) made an extensive study on the culture characteristics of the organism and found 5 per cent rabbit blood agar as the best solid medium for the growth of the organism in 48 h at an optimum temperature of 28°C and *p*H 7.2-7.6. This growth was generally obtained by heavy inoculum and always needed a lag phase. So the knowledge of nutrition of the organism was considered essential by the author (Seal and Mukherji, 1950) for growth with small inoculum. Various explanations were put forward for this lag phase by Schutze and Hassanein (1929), and Rao (1940). It was found that some nutrients and enrichment substances tried to facilitate better growth of organism with small inoculum. These were organ lysates (Samsonov, 1935), special peptones (Girard and Neel, 1946), bubbling air through broth (Devignat, 1942) and rat meat or rat liver (Yu, 1949).

In the preparation of both plague vaccine and antiplague serum Sokhey strongly felt the necessity of intensive experimental study on the cultural, morphological, serological, and other characteristics of the plague organism, its chemical antigenic structure and the biological and serological behaviours of dissociation, without which an effective measure for the control of the disease was not possible. Sokhey (1936, 1939, 1940) and Sokhey and Habbu (1943) made some useful contribution to the methods of isolation, *p*H of the culture medium, temperature of incubation, classification and preservation of organism, preparation of vaccine and antisera, test of virulence and mouse-protective test with the Institute's special in-bred white mouse.

The most disturbing handicaps in the study of the organism and its serological and chemical antigenic structure were the salt-instability of the

suspension of the organism causing auto-agglutination and the use of media containing digested meat and unbroken protein moiety. The earlier workers including Rowland (1910) and Sokhey (1940) found immunising antigen to be a nucleo-protein in nature and it was found difficult to isolate this substance in a comparatively pure form in a medium containing non-specific protein. Shrivastava (1940) tried gelatin hydrolysate medium to overcome this difficulty but his work remained incomplete. At the time of undertaking special studies on the problem in 1940, the author noted the following lacunae in the knowledge about plague organism:

(i) Absence of good liquid medium for the preparation of liquid antiplague vaccine.

(ii) Absence of effective serological method for identification of the organism, as with the available methods of culture the organism was always found auto-agglutinable. The only method left for identification was the mouse or guineapig inoculation test, which was time-consuming and required a well-equipped and organised laboratory to undertake the job. This identification being based on virulence of the organism killing the test animals it failed if the organism was avirulent or partially virulent. Thirdly, *Y. pseudotuberculosis*, an organism pathogenic to rodents also having a cross-antigenic relationship with the plague organism, it became difficult to differentiate the two organisms.

(iii) Absence of serological classification of organism due to the auto-agglutinating nature of the organism grown on the available media.

(iv) Lack of knowledge on chemical antigenic structure and of the identification of specific protective substance.

(v) In absence of methods for isolation and identification of the specific protective or immunizing substance, the only method of assessment of titre of antiplague sera was time-consuming mouse protection test—a great disadvantage in the production of therapeutic serum in horses at that time.

(vi) In the absence of precise knowledge about the organism and efficient culture medium, the potency of the antiplague serum could not be improved; and

(vii) For the same reason, knowledge about the epidemiology of the disease remained incomplete.

Every aspect of the above problems was studied by the author who finally succeeded in solving all of them. The details are briefly described below:

Nutrition of Plague Bacillus

The nutrition of plague bacillus was the essential prerequisite for the plague studies because on this depended the culture, morphological, biochemical, antigenic, virulence and other differential characters. It does not grow in ordinary agar or broth medium unless the organism concerned was seriously degenerated. Certain additional nutritive and accessory growthfactors were necessary for its full development and antigenicity (Rao, 1939, 1940).

This was also the basis of author's own work in some of his investigations (Seal and Mukherjee, 1948, 1950; Seal, 1950; 1951a; 1953) as it was found extremely important from the point of view of maintenance of the organism without losing its virulence and antigenicity for the preparation of vaccine.

A preliminary quantitative analysis of the virulent *Y. pestis* reveales that it was composed of the following elements *viz.*, C, H, N, O, S, P, Na, K and traces of Mg and Ca. Casein hydrolysate medium of Seal and Mukherjee (1950) provided not only these elements but also the three essential aminoacids—prolein, phenylalanine and cystine-besides other aminoacids except tryptophane, as propounded by Rao (1940) thus supplying C,H,O,N,S, and P. Subsequently, Jackson and Burrows (1956) noted the virulence enhancing effect of iron on the relatively avirulent mutants, which the author had included in his casein hydrolysate medium along with traces of Ca, P, and Mg and small amount of liver extract. One of the criteria of a nutritive media is the virtual absence of the so called lag phase as noted in the casein hydrolysate enriched broth described below:

New Culture Media

Hydrolysate of Casein as a Fluid Medium for the Growth of Yersinia pestis

To facilitate isolation of specific protein in pure form a protein-free casein hydrolysate broth was prepared by a modified method of Mueller and Johnson's (1941) hydrolysate of casein and thereafter enriching it with certain nutritive substances including liver extract. Critical experimental studies were carried out by Seal (1943) and Seal and Mukherjee (1950) on the following aspects before arriving at the final composition of the medium. These include method and period of digestion of casein to obtain the maximum amount of precipitable amino acid and ammonia nitrogen, total nitrogen, growth of *Y. pestis* according to period of digestion, mineral contents and requirements (chloride, iron phosphate, calcium and magnesium) and the effect of additional nutrient in the form of liver extract for enrichment of growth.

The optimum period of digestion of casein for the preparation of the crude hydrolysate medium was found to be 72 h as shown by the growth curve of the organism (Fig. 29).

On comparative estimation of nitrogen distribution in acid-digested mutton broth, gelatin and casein hydrolysates, the best result was obtained with the casein hydrolysate medium in respect of total and amino-N/ml and less tungstate precipitable N. Casein hydrolysate broth also gives the highest growth of *Y. pestis* (320 million/ml against 150 or 160 million) by the other two media but it did not exceed the growth obtained in nutrient broth (400 million/ml). Hence further improvement of this crude hydrolysate broth was obtained by adding a small amount of liver extract.

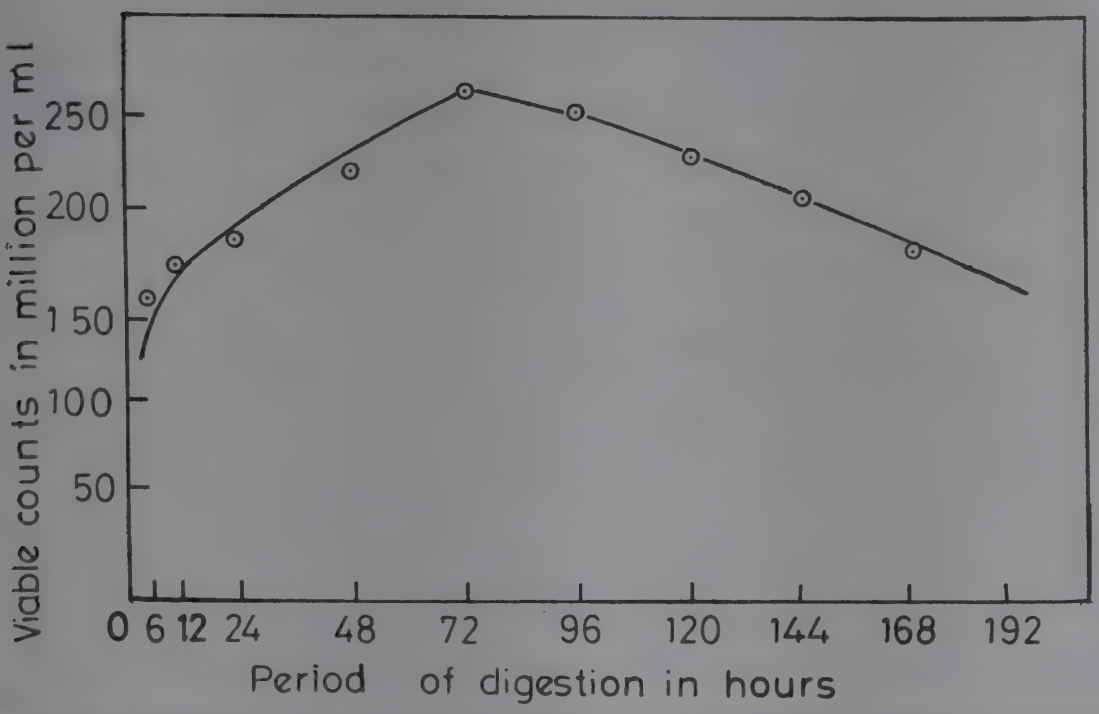

Fig. 29. Growth curve of *Y. pestis* in relation to the period of digestion of casein.

Rate of Growth of Y. pestis and the Lag Period

The best criteria of a good medium are abundant growth and minimum or absence of lag period. To test the efficiency of the above casein hydrolysate broth medium with or without liver extract it was inoculated with 6 h seed culture which was prepared in the following manner:

After two consecutive subcultures in nutrient broth at intervals of 48 h the third subculture was incubated for 6 h at 28°C and was used as a seed. The sowing was done with 0.1 ml of 2×10^3 dilution of broth to 9.9 ml of the experimental broth. The medium was then incubated at 28°C. Viable counts were made after varying intervals of incubation period beginning from 1/2 to 54 h. A simple protocol of the results obtained is given in Table 26.

Table 26. Viable counts/ml of growth of *Y. pestis* in casein hydrolysate (CH) medium in relation to the period of incubation

Period of incubation, h	CH basal	CH with liver extract	Period of incubation, h	CH basal	CH with liver extract
(Seed)	556	420	18	28×10^8	226×10^8
1/2	—	595	24	155×10^8	286×10^4
1	858	645	30	85×10^4	25×10^6
2	615	1.005	36	77×10^8	65×10^6
3	750	Not done	42	872×10^5	106×10^6
6	2,800	4,450	48	98×10^6	Note done
9	4,400	Not done	54	1075×10^5	193×10^6

CH—casein hydrolysate.

Table 26 shows that the casein hydrolysate basal medium yielded quite a good growth practically without any lag period. This was further improved by adding liver extract. This result was obtained in spite of frequent disturbance during withdrawal of samples for viable counts.

Specific Soluble Protein of Y. pestis in the Casein Hydrolysate Medium (Preliminary Test)

To test the efficiency of the medium for isolation of the specific soluble protein of plague bacillus the final medium was distributed in four flasks. After inoculation with the *Y. pestis* culture, the flasks were incubated at 28°C and removed at successive intervals of one week. The filtrates were then separately saturated with Na_2SO_4 at 37°C and the precipitate collected upon a filter paper, dissolved in water and dialysed till sulphate free and finally dried in a dessicator over P_2O_5. The highest yield was from the sample removed at the end of 3 wk (Fig. 30). Each of these was then dissolved in 0.85 per cent NaCl solution at *p*H 7.2-7.4 so as to give 1 mg of substance per ml. Dilutions between 4000 to 1,28,000 were made of each specimen (the actual dilution was determined by nitrogen estimation) and 0.1 ml of each was mixed with 0.5 ml of 1 : 10 dilution of a potent antiplague serum

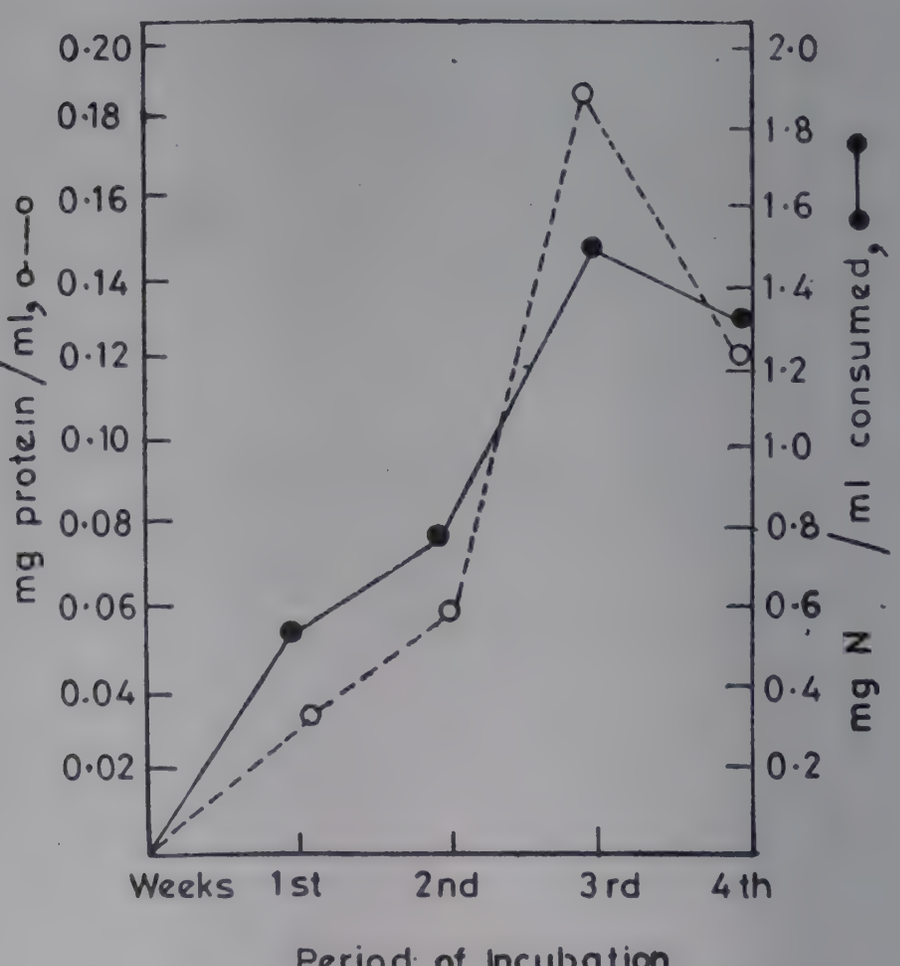

Fig. 30. Yield of specific soluble protein (crude) and nitrogen consumption in mg/ml of CH broth in relation to the period of incubation.

(H 23) incubated at 37°C for 2 h and then left overnight in the refrigerator. The results as read next morning showed that the crude protein isolated from casein hydrolysate medium gave a specific precipitation reaction in fairly high dilution of 1 : 128,000 with small improvement with period of incubation up to 4 weeks. Thus, this preliminary test gave highly encouraging results.

Comparative Mouse-Protective and Toxic Doses of the Vaccines Prepared in Casein Hydrolysate and Other Media

The mouse-protective doses of the usual Haffkine broth vaccine and of vaccines prepared from the 3 days' as well as 4 weeks' growth in casein hydrolysate broth at 28°C and from 3 days' growth on agar at 37°C were estimated by Sokhey's method at the Haffkine Institute, Bombay.

The results showed that the vaccines prepared in casein hydrolysate broth were more potent than Haffkine broth vaccine and the potency remain unaffected by storage even at 37°C for 8 months.

Toxicity of Different Types of Plague Vaccine

One of the objectionable features of Haffkine plague vaccine has been its toxicity. As a matter of fact the original conception of potent vaccine was to depend upon its toxic action. The results of determining the toxic dose of the three different vaccines are given in Table 27.

The toxicity of casein hydrolysate broth vaccine is of low order. The medium also gives negative biuret reaction and intradermal injection does not produce any adverse reaction. The results of experimental work in relation to this newly developed casein hydrolysate medium are given above rather in detail because all the subsequent development of newer knowledge that led ultimately to deal with the problem adequately depended on the studies based on plague bacilli cultured in this medium.

Casein Hydrolysate Agar as a New Medium for Growth of Yersinia pestis and Allied Organisms

In an attempt to find a suitable solid medium for enumeration of viable

Table 27. Toxicity and mouse-protective doses of three different plague vaccines

Type of vaccine	Mouse protective dose, ml	Toxic dose, ml
1. Haffkine broth vaccine	0.006	0.2
2. Casein hydrolysate broth (1000 million organisms/ml of 3 days' growth)	0.0053	0.8
3. Agar vaccine (1000 million organisms/ml)	0.0048	1.8

plague organism Sokhey (1939) recommended 5 per cent rabbit blood agar as the choice for growing virulent plague bacilli. The need to replace blood agar by a suitable solid medium was required for 2 reasons *viz.*, (*i*) to avoid rabbit blood in the culture media which may cause adverse reaction with the vaccine prepared from such a medium for human immunisation or for the production of antiplague serum in horses; and (*ii*) it would eliminate procurement of rabbit's blood on a large scale. Casein hydrolysate broth, being fully nutritive for plague bacilli opportunities, were taken to prepare solid media out of this CH broth. The advantages of such a solid medium were: (*a*) facilitating colony count; (*b*) preservation of virulent *Y. pestis*; (*c*) preparation of bacterial suspension for quantitative agglutination, virulence and animal protection test; (*d*) preparation of antiplague vaccine, and (*e*) facilitating isolation and study of chemical antigenic structure of the plague and allied organisms such as, specific proteins (from the autolysates) and polysaccharides. It will be found later that all the above advantages were fully utilised in acquiring advanced knowledge needed to eradicate the disease.

Viable Counts of Y. pestis in Casein Hydrolysate (CH) Agar

Roux bottles containing CH Agar were inoculated with 12-15 ml of 48 h seed culture and spread well over the surface of the medium and incubated at 37.5°C for 48 h. The final yield of vaccine was about 10 times that of ordinary nutrient agar. A viable count of *Y. pestis* grown in casein hydrolysate agar was compared with those of organisms grown on nutrient agar by subculturing on 5 per cent rabbit blood agar.

Studies on *Y. pestis*, *Y. pseudotuberculosis* and their Variants for Vaccine Production

Growth, Morphology, Salt Stability and Biochemical Reactions

Prior to the author's work divergent claims were made by different plague workers on the use of strains of *Y. pestis* for the production of plague vaccine. One group including Sokhey (1939) emphasised the use of virulent strain and another including Otten (1936), Girard and Robic (1934) advocated the use of live avirulent plague strain. There was also another controversy on the use of 'smooth' and 'rough' strains for the preparation of prophylactic vaccine arising out of Schutze's observation in 1939 that neither the virulent strain had any superiority over avirulent strain nor the smooth over the rough colonies.

Wats, Wagle and Puduval (1939) stated that the presence or absence of specific soluble substance did not depend upon smooth or rough colonies but as a general rule a strain producing majority of rough colonies was likely to be poor in specific soluble substance. Bhatnagar (1940) also suggested that a quantitative relationship did not exist between the type of colonies and virulence. Both Bhatnagar (1940) and Jawetz and Meyer (1944 b) observed virulent strains to produce largest number of dew drop colonies

and its variant, the opaque colonies, with transparent fringe. The avirulent strains, on the other hand, produced smooth circular type of colonies.

Pollitzer (1936) in his comprehensive review has mentioned the diverse and puzzling discrepancy of opinions of the various workers regarding dissociation of plague bacillus. According to him most of authors have stated that the plague bacilli were usually met within rough state, while others had contrary opinion. Pollitzer (1936) therefore recommended further studies on the subject including exhaustive investigation of quite fresh material. Since the actual character of the colonies may be influenced by the media in which the organisms are grown, it was of interest to study their colonial, morphological, bio-physical and biochemical characters in different media with a view to find out if there is any relationship between any of these characters and virulence or their antigenic (immunogenic) qualities.

For this study the following strains were selected: (*i*) virulent plague strains (Bhatnagar's 1940 classification) —6 strains isolated from human plague cases and 16 other strains from the same outbreak to confirm the results of sugar fermentation test; (*ii*) Avirulent protective plague strains EV of Girard and Robic (1934), Tjiwidej smooth and Tjiwidej rough of Otten (1936) and 53/Hav of Haffkine Institute; (*iii*) Avirulent non-protective-5 strains; (*iv*) *y. pseudotuberculosis* rodentium-4 straints originally from the National Type Culture Laboratory, London. All these strains were maintained in the first sub-culture on 5 per cent rabbit blood agar and maintained at $\pm$ 4°C in sealed tubes. For all tests sub-cultures were made freshly from the original tubes. The sub-cultures for study purposes made in the fluid media were incubated at 28°C for 48 h and those on solid media at 37°C for 48-72 h. All organisms were tested for colony appearance, salt stability, morphology, motility, fermentation of sugar, indol reaction, methyl red test, V-P test, H_2S, methylene blue and litmus milk reactions. Since all the members of the Pasteurella group of organisms including *Y. pestis* reduce nitrates, from ammonia and give positive catalase test these were not performed. To study colonial appearance, salt-stability and morphology each strain was grown on four different media namely, (*i*) Acid-digested mutton agar (A.D. agar); (*ii*) 10 per cent horse serum agar; (*iii*) 5 per cent rabbit blood agar; and (*iv*) nutrient broth (broth prepared by digesting mutton with HCl was used as the base for the first medium, the rest being prepared according to the standard method).

The motility was tested by culturing the organism in three ways namely, (*i*) in semi-solid agar ($\frac{1}{2}$%) at 37°C for 48-72 h developed by Seal (1952); (*ii*) in 1 per cent peptone water at 28° and 37°C for 24 and 48 h; and (*iii*) in nutrient broth at 28° and 37°C for 24 and 48 h. Control culture of known motile and non-motile organisms were simultaneously done. For testing the sugar fermentation reactions sugars of 19 types were used and besides using the standard method a simplified sugar medium developed by Seal (1952) was also used.

Indol reaction was tested both by residual reagents and Gore's (1924)

technique, as well as a new technique developed by Seal (1952). To test the reduction of methylene blue 1 drop of 1 per cent solution of methylene blue was added to 10 ml of 24 h culture in nutrient broth at 28°C and incubated the tube for a further period of 24-48 h at 28°C. For other tests the standard techniques were employed. To prove the presence or absence of envelop substance new method was used besides India Ink method by the author (Seal, 1960).

Culture Characteristics

(*i*) General growth: With all strains the heaviest growth was obtained on rabbit blood agar, good on serum agar and poor on AD agar. More abundant growth was, however, obtained on AD agar for pseudotuberculosis and avirulent non-protective plague bacilli. While a large inoculum was required for growth on AD agar, a small quantity was found sufficient for growth on rabbit blood agar. The growth in all cases was better at 28°C than at 37°C.

(*ii*) Colony appearance: The colony appearances were more or less uniform on rabbit blood agar, variable on serum agar and irregular and variable on AD agar.

(*a*) On AD agar—It was observed that virulent and avirulent plague strains give poor growth on AD agar. Two types of colonies were generally noted. The most common type was a flat translucent colony with central capacity or thickening and peripheral lighter fringe while the other was represented by round opaque colonies with or without the outer fringe. The avirulent non-protective plague and pseudotuberculosis strains showed mostly the rugged flat opaque colonies and very few with lighter outer margin.

(*b*) On serum agar—The distinction between the protective virulent and avirulent and the non-protective plague organisms was more marked on this medium than on AD agar. The former generally produced more or less smooth, flat and translucent colonies somewhat mucoid or viscous in nature with or without central thickening and lighter outer border. The colonies of the non-protective plague organisms, on the other hand, were comparatively larger, generally rough or rugged and more opaque with or without thinner and irregular spreading margin, some of them being actually circumvalate in their appearance. The pseudo-tuberculosis strains showed both smooth and rough types of colonies.

(*c*) On rabbit blood agar—The colonies on this medium were even more sharply defined than those on serum agar. The virulent *Y. pestis* showed mainly two types of colonies *viz.*, (*i*) small, smooth, slightly glossy, convex, dew drop-like translucent colonies; and (*ii*) slightly bigger translucent colonies with smooth or ruffled surface and a thin outer border and tendency to haemodigestion. The colonies were somewhat viscous and thus appeared sticky. Dew drop type appearance was seen between 24-48 h of incubation. The avirulent protective plague strains showed medium-sized flat smooth colonies with

central thickness, but no tendency to haemodigestion. The exception was E.V. strain which showed glossy convex colonies without outer thin fringe. It resembled the dew drop colonies of virulent plague strains, gave extremely poor growth at 37°C but easier and profuse growth at 28°C or at room temperature (31.5°C). The avirulent non-protective plague strains, on the other hand, showed generally large and comparatively more opaque and rugged type of colonies with or without faint and irregular outer fringe. The colonies of pseudotuberculosis strains also closely resembled this latter type but were often mixed with some smooth colonies. The colony characters in casein hydrolysate agar were the same as noted on serum agar and hence not separately shown.

(*iii*) Growth in nutrient broth: The smooth virulent plague strain showed a homogenous light turbidity in 48 h at 28°C with a comparatively heavy growth at the upper part of the medium. Similar results were obtained with the avirulent protective plague strain except Tjiwidej S and R strains which showed finely granular type of growth slightly thicker at the upper part. If left undisturbed the above strains did not give any deposit at the bottom within 48 h. The avirulent non-protective plague strains, on the other hand, produced granular growth in broth and formed deposit at the bottom and often at the sides of the tube with or without heavier growth at the top within 48 h at 28°C. The growth of pseudotuberculosis was greater and more or less resembled with that of avirulent non-protective plague strains. The growth at 37°C was much poorer than at 28°C with all plague strains but at this temperature of incubation larger envelope substance was shown by the virulent and avirulent protective plague strains only.

Morphological Characters of the Organism

Y. pestis is a Gram-negative organism short and ovoid in shape showing bi-polar staining. Some workers confused spore-bearing organisms with plague bacilli. Others also described various types of involution forms like chain, branching, filamentous, ring forms *etc.* (Haffkine, 1897; Hankin and Leumann, 1897; Sata, 1901; Bouffard and Girard, 1927; Smidt, 1929; Korobkova, 1937; Wayson and Mc-Mohan, 1944; and Roux and Mercier, 1946). On scrutiny these involution forms may be attributed to the nature of cultural media, late isolation from carcasses of rodents died of plague infection, bacteriophage, low temperature of incubation and effects of drug treatment prior to collection of specimens for isolation of the organism by culture methods. Devignat and Schoetter (1942) reported that repeated passage of plague bacilli through aerated media was apt to lead the appearance of involution forms and resulted in a loss of virulence of the strains. The bipolar character of the plague bacillus is due to the presence of a chromatic corpuscle near the poles which takes part in the cell division according to Wei *et al* (1948), the multiplication taking place through a process of gemmation.

Morphological Appearance of the Organism After Staining

The virulent plague organisms grown mainly on rabbit blood agar were

pale staining (by carbol-thionin) ovoid organism (Fig. 31). A few of them also showed somewhat long and thread like shape (particularly when grown on AD agar). These were the general characteristics of avirulent non-protective plague strains. The latter however were more deeply stained and often took the rod-shaped bacillary as well as pleomorphic forms.

Capsule and Envelope

According to Kitasato (1894) plague bacillus is a capsulatad organism while Rowland (1914) postulated that the plague bacillus possessed a slimy 'envelope' other than capsule. This was supported by Schultze (1939), Bhatnagar (1940) and Seal (1951a). Sokhey (1940) postulated that capsule was present under all circumstances and envelope seemed to be only an unstained capsule. Amies (1951), on the other hand, confirmed through studies in wet India ink preparations under dark-ground illumination that the so-called envelope of *Y. pestis* was nothing more than a particularly well-developed bacterial capsule. According to Chertnik (1940), *Y. pseudotuberculosis* has no capsule or membrane antigen. This observation was supported by Schutze, Bhatnagar and Seal. Otten (1936) also found avirulent Tjiwidej strain uncapsulated. Seal (1960a) carried out some intensive study and developed an ingenuous method of staining to demonstrate both capsule and envelope in virulent plague bacilli.

By this technique virulent *Y. pestis* grown at 37°C on rabbit blood agar

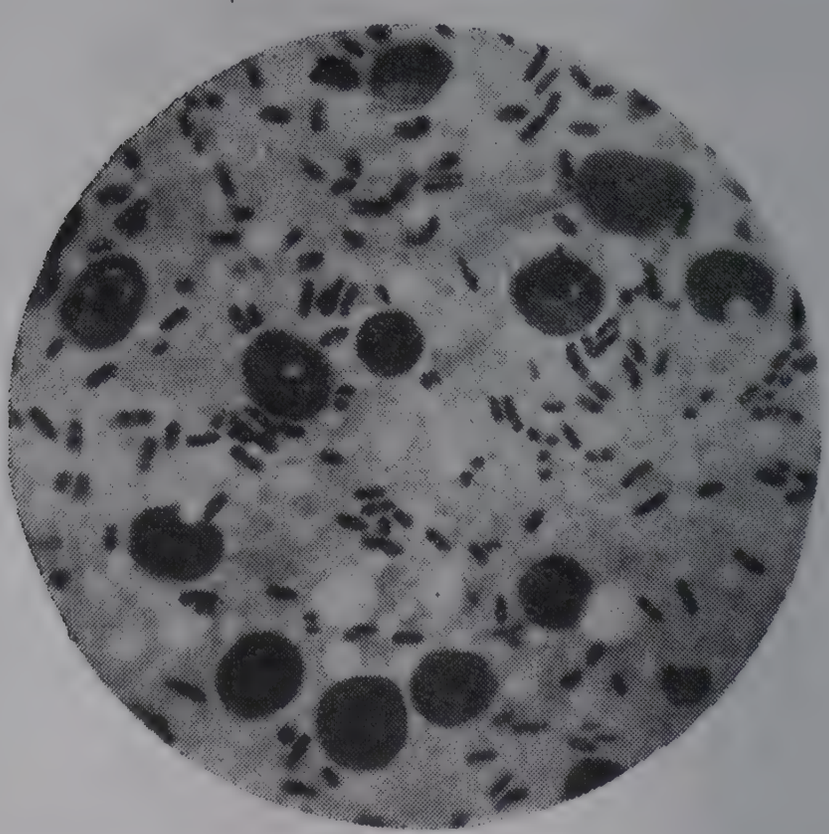

Fig. 31. Smear from enlarged lymph gland from a case of plague; numerous *P. pestis* are seen as ovoid bacilli with characteristic bipolar staining. Film treated with Leishman's stain × 1,000.

showed the largest amount of envelope substance but it was smallest on strains grown on A-D agar. At 28°C also the envelope substance was almost negligible in all media and particularly A-D agar. While the protective avirulent plague strains, like Java, Madagascar (EV) strains also contain the envelope substances, the avirulent non-protective plague as well as *Pseudotuberculosis* strains did not show the presence of envelope substance in any of the media incubated either at 28° or 37°C.

Other Characteristics of the Organism

(*i*) *Motility*

For motility test stab culture in semi-solid agar incubated at 25°C or at slightly low temperature was found convenient (Seal, 1952). A characteristic tree-like growth was given by the pseudotuberculosis strain as an evidence of motility, whereas the non-motile plague strains remained as a single streak. Better results were obtained even in broth or peptone water when the temperature of incubation was the same as that in case of semi-solid agar.

(*ii*) *Salt Stability*

It is well-known that the plague strains ordinarily produce an unstable suspension in normal salt solution but in the present study all plague strains gave smooth colonies when cultivated on 5 per cent rabbit blood agar or casein hydrolysate agar for 24 or 48 h at 37°C (better at 28°C). This method of preparation of live suspension completely obviated the difficulties in carrying out agglutination test.

(*iii*) *Biochemical Reactions*

All plague strains gave negative indol and V.P. reactions and did not reduce malachite green or methylene blue (1 : 50,000), while the pseudotuberculosis strains gave negative indol and V.P. reactions but gave positive H_2S test, reduced malachite green and methylene blue and changed litmus milk to slightly alkaline reaction. All strains were methyl red positive but the typical red reaction was given by pseudotuberculosis and avirulent non-protective plague strains and yellowish red reaction by virulent and avirulent protective plague strains.

(*iv*) *Action on Carbohydrates*

Altogether 23 virulent *Y. pestis*, 4 avirulent protective *Y. pestis*, 3 aviru- lent non-protective *Y. pestis*, 4 *pseudotuberculosis rodentum* and 2 *Y. avisepticus* strains were tested against 19 sugars for their fermentation reaction. Of these, only 10 sugar including glucose, galactose, levulose, arabinose, xylose, maltose, trehalose, mannitol, dextrin and salicin were fermented by plague strains forming acid only. Galactose was fermented in 48 to 72 h and salicin in 72 to 96 h. The avirulent protective Madagascar strain (EV) and the avirulent

non-protective plague strain TRU failed to ferment galactose. The sugars that distinguish pseudotuberculosis from plague strains were rhamnose and iso-dulcitol which produce acid with former but not with the latter strains. *P. avisepticus* did not ferment maltose and rhamnose but fermented saccharose and sorbitol, thus differentiating it from both plague and pseudotuberculosis strains. To distinguish the pseudotuberculosis type strains from one another the three sugars *viz.*, raffinose, trehalose and dextrin were useful, dextrin being fermented by PR/I, trehalose by PR/II and raffinose by PR/III and both raffinose and trehalose by PR/IV (Table 28).

Though a considerable amount of early work had been done with sugar-containing media a definite proposal to use them for differential purposes was made by Colas-Belfour in 1926. Bezsonova (1930), however, recommended that the sugar media worked better with rhamnose containing base instead of glycerol, as also endorsed by Girard (1950b). Two other substances which have been used for differentiation between plague and pseudotuberculosis strains are melbiose which is specially attacked by pseudotuberculosis and glycogen by plague bacillus (Prade, 1940 and Russo, 1940).

Table 28. Differential sugar fermentation reaction by different strains

Strain	Sugar								
	Maltose	Rhamnose	Raffinose	Saccharose	Trehalose	Inulin	Dextrin	Sorbitol	Isodulcitol
Plague strains	A	O	O	O	A	O	A	O	O
PR/I	A	A	O	O	O	O	A	O	—
PR/II	A	A	O	O	A	O	O	O	—
PR/III	A	A	A	O	O	O	—	O	A
PR/IV	A	A	A	O	A	O	—	O	A
P. avisepticus	O	O	O	A	O	A	O	A	—

O = not fermented; A = fermented; — = not done.

Proposed subgroup	Test with glycerol	Production of nitrous acid	Remarks
1. *Y. pestis* var *orientalis*	—	+	Oceanic race (Berlin and Borzenkov, 1938)
2. *Y. pestis* var *antique*	+	+	Found in Central and North Asia and in Central Africa
3. *Y. pestis* var *mediavevalis*	+	—	Found in South-East Russia

Other Biochemical Functions

(*a*) *Alkali Formation*

The growth of plague bacillus in broth stopped between 5-11 wk due to exhaustion of the medium (Haffkine, 1899). Bannerman (1905) showed it to be due to alkalinity in the broth corresponding to 1.5 to 2 per cent N/NaOH in 6-8 wk. Naidu and Jung (1927) showed that even after reaching the maximum alkalinity by neutralisation of the medium it resulted in further growth of the bacilli. Normally plague bacilli do not curdle milk nor produce hydrogen sulphide but reduce nitrates (Fusco, 1927; Schutze, 1929; Gore, 1930; and Girard, 1940).

(*b*) *Enzymic Activity*

Herbert (1949) found that plague bacillus grown on amino acid glucose medium or an agar plate produces catalase (haematin-protein) even if no haematin was added to the medium. Rockenmacher (1949) considered the catalase activity to have been associated with virulence. The organism also shows ribo-nuclease activity (Woodward, 1944) and marked fibrinolytic activity particularly for rat and guineapig fibrin (Madison, 1936). Both virulent and avirulent plague strains also produce coagulase for rabbit and guinea-pig plasma (Jawetz and Meyer, 1944a) while Fauconnier (1950) confirmed that the plague strains did not show any urease activity and this property could be used for differentiating the two organisms.

(*c*) *Race Differentiation by Action on Glycerol*

Bezsonova (1930) noted that plague strains, isolated in South-East Russia, Manchuria, Mongolia, Transbaikalia and Turkistan, acidified glycerol media whereas strains from India and Italy failed to do so. Based on this finding Kurauchi (1937) and Berlin and Borzenkov (1938) distinguished two races of plague bacilli—a glycerol-positive race found in the central plateau of Asia and adjacent territories and a glycerol-negative race (Oceania race) found in the peninsulas and islands of the tropics into which plague was introduced by sea-route. Glycerol-positive strains have also been isolated in certain parts of Africa namely, Belgian Congo, Uganda and part of Kenya where plague is of long-standing prevalence like that in Central Asia. But introduction of plague by sea-route into the Union of South Africa led to the isolation of both glycerol-negative and glycerol-positive types and hence has been described as a transitional area. Accordingly, it is presumed that the plague strains during 'Black Death' or Philistine or Justinian outbreaks were of glycerol-negative type. According to Pollitzer (1954) the change might have taken place during the transfer of plague infection from wild rodent to *Rattus rattus* as in Burma. It is, however, doubtful if there is any fundamental difference between the two races. Devignat (1951a) proposed the following classification for plague bacilli:

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Dissociation and Mutation

The significance of the two forms of plague bacillus Smooth and Rough was interpreted differently by different workers. Bezsonova and Lenskaia (1931) considered 'Rough' as the usual and virulent type of plague bacillus, while Pirie (1929) distinguished between a normal smooth and mutant rough type. Pirie's work was supported by Rachinski (1930) and Wats and Puduval (1940). Otten (1936), Girard (1941a) and Macchiavello (1941b) issued a caution note in strictly applying the definition of dissociation in the same sense as applied in other bacterial genera. Some strains like avirulent EV Madagascar strain during subculture was found to form yellowish pigment (Korobkova, 1940; Devignat, 1943; and Girard, 1958) often with loss of antigenic and immunogenic properties of parent strain. It was suggested that the change might have been due to some external action by bacteriophage or by contaminants.

Virulence and Toxicity of Plague Bacillus

Virulence and toxicity of plague bacillus though not synonymous they are complementary to cause the death of the infected animal or man. By virulence is meant not only invasiveness but also the means of bringing out ultimately a fatal termination of the disease due to the toxicity of the organism. Generally speaking, virulence of plague bacillus is normally high as shown by Barber (1912) in his classical experiment in guineapigs and monkeys by inoculation of supposedly single plague bacillus. Otten (1933) used 6-12 plague bacilli for standard virulence test in mice. Although virulence is fairly stable when the organism is maintained properly, there is a fair evidence of change of virulence in artificial culture and prolonged keeping in the laboratory (Revenstorf, 1909; Macchiavello, 1942). Plague strains of low virulence has been noted in natural outbreak and in chronic plague in rats (Pirie, 1936; Meyer *et al* 1937).

(*a*) *Test of Virulence*

Various workers recommended their respective methods for testing the virulence *e.g.*, rubbing a standard dose of plague culture on the shaved skin of guineapigs (Kolle and Martini, 1902) or rats (Sokhey, 1936). Rowland (1910) injected the culture subcutaneously into rats. Finally Sokhey (1939) devised the standard method at the Haffkine Institute, Bombay, by injecting broth-culture of known counts by progressive dilutions each 1/10th the strength of the preceding one and subculturing the same or blood agar, the number of colonies grown indicating the number of organisms contained in the dilutions. Each of these dilutions was injected into a group of 5 Haffkine Institute inbred white mice. He noted that 5-10 organisms were enough to kill 100 per cent mice. Results were expressed as the smaller number of organism of a given strain of *Y. pestis* which when administered subcutaneously killed approximately 100 per cent of the animals as estimated by statistical method within 3-11 days.

(*b*) *Maintenance of Plague Strains*

The virulence is maintained by passage through animals-mice (Sokhey, 1939), and storage at low temperature (4° ± 2°C) on rabbit blood agar slab culture or by freeze-drying of the organism suspended in gum acacia or milk substrate at *p*H 7.0-7.2 (Seal, 1942). The dried cultures are preserved at 0° ± 1°C and regenerated by dissolving in broth or distilled water and subcultured in 5 per cent rabbit blood agar.

(*c*) *Serological Properties and Virulence*

According to several workers like Schutze (1939), Wats *et al* (1939) and Jawetz and Meyer (1943), it was impossible to distinguish virulent from avirulent and smooth strains from rough strains by means of serological test. But it was made possible (Seal, 1951a-e) and has been described in Chapter IX.

(*d*) *Attenuation of Plague Bacillus*

The process of attenuation is either natural or enforced by subjecting the strains to adverse environmental conditions. Otten (1941) was able to obtain avirulent subcultures by simple colony picking from virulent cultures of *Y. pestis*, which he later used for the preparation of live avirulent plague vaccine. Repeated subculture or prolonged maintenance in artificial media often leads to loss of virulence (Girard and Robic, 1936; Sokhey and others, 1939; Jawetz and Meyer, 1943). Other workers cultivated the organism in alcohol broth to obtain avirulent form. Earlier Bergess (1930) attenuated the virulence by passage through immune animals. Pokrovskaya (1935) did it by bacteriophage. The purpose of these procedures was to obtain an attenuated organism without loss of immunogenic properties, to be used as live vaccine such as Tjiwidej strain by Otten (1936) and EV Madagascar strain by Girard and Robic (1936).

Toxin of Plague Bacillus

It was Rowland (1912) who prepared toxic nucleoprotein in 1912 by extracting agar-grown plague cultures killed with chloroform, with anhydrous sodium sulphate. This extract administered in doses of 0.05 to 0.1 mg were fatal to rats within 18 h while the same used in doses of 0.001 to 0.01 mg afforded protection against plague infection. Petrie (1929), however, believed that all preparations used for active immunization against plague contained toxin or toxoid or a mixture of these antigens. Markl (1901), Kessel and Overbrook (1901), Dieudonne and Otto (1928) suggested that the toxin of *Y. pestis* might represent a mixture of metabolic products of the living bacteria and of endotoxic substances set free after their disintegration. Absence of gluco-lipid complex found in endotoxin of numerous other Gram-negative organisms led Girard and Sandor (1947) to suggest that *Y. pestis* endotoxin resembles exotoxin and that it could be transformed into toxoid and was rather thermolabile. However, most workers are convinced that the plague

bacillus has an endotoxin only, as originally postulated by Rowland (1912).

Various methods have been used to extract the toxin from plague bacillus, the extracts being nucleoprotein. Baker *et al* (1947) pointed out that it had not been possible to isolate this toxin fraction in a state of approaching chemical and immunological purity, all preparations being contaminated with immunogenic fraction I in sufficient quantities to produce antibodies to the latter in rabbits. They could separate the toxin only by serological techniques, the resulting absorbed extract did not show decrease in toxicity and the antiserum produced in rabbits was capable of neutralising plague toxin. But this antiserum was devoid of protective value for mice and did not agglutinate plague bacilli significantly. Toxic lysates were also obtained by Girard (1950 a and b) by adding bacteriophage to a three day old culture of EV strain and filtering after 7 h, 0.05 ml of these lysates injected intraperitonially killed mice in 10-12 h. But he failed to obtain toxic filtrates from broth culture incubated at 28°C before 6th to 9th day.

The transformation of the plague toxin into toxoid is easily effected by adding 0.34 per cent formalin to plague culture at 37°C for a few days. According to MacConkey (1912) the toxin in old autolysed solution of plague nucleoprotein undergoes partial change into toxoid and this substance stimulates the production of plague antitoxin in horses.

Antigenic Structure of Plague Bacillus

The first attempt to study the chemical and immunogenic fraction was made by Rowland (1910) repeated and confirmed subsequently by Morison *et al* (1924). According to these workers plague bacillus appeared to have two types of protein, one soluble in distilled water or saline and containing the immunogenic and toxic substances and the other insoluble and possessing no antigenic or toxic properties. The acetone-extracted substance referred to earlier (Bakeg *et al* 1947) also yielded a water soluble and water insoluble fractions with the difference that water insoluble fraction, though inert so far as mice and rats were concerned, proved of high immunogenic value for guineapigs when tested as an alum precipitate. Precipitation with ammonium sulphate at different concentrations yielded two fractions IA and IB, both fractions developing immunity in mice, rats and monkeys but not in guineapigs (Meyer, 1950 a). In a subsequent observation Baker *et al* (1952) stated that the difference between IA and IB fractions was due to the loss of carbohydrate moiety (present in IA) in the fraction IB, and that these fractions were formed by virulent plague bacilli and salt-stable avirulent form when cultivated at 37°C. According to Bhatnagar (1940) it is the 'envelope' substance which bestows the salt-stability and those without it floculate into granular clumps (Amies, 1951).

(*a*) *Active Immunity Against Plague*

In 1895, Yersin *et al* showed, for the first time, that rabbit could be immunized against plague by repeated inoculation with suspensions from agar

culture killed at 58°C for one hour. About a year later Haffkine (1897 a and b) obtained identical results with broth culture of *Y. pestis* sterilised by heating. Since then large scale preparation and use of both agar grown and broth grown killed and even live vaccines had been used for protection against plague. The two types of vaccines now used are (1) killed and (2) live avirulent (Otten's).

(1) *Killed Vaccine*

The types of killed vaccines used are: (a) Haffkine vaccine; (b) Agar-grown vaccine; (c) Pseudotuberculosis vaccine; (d) Lipo-vaccine; (e) Sugar vaccine; (f) Precipitated vaccine; and (g) Chemically prepared extracts of *Y. pestis*.

(*a*) *Haffkine vaccine*: This was originally prepared in meat broth but since the introduction of casein hydrolysate liquid medium by Seal and Mukherjee (1943, 1950), this vaccine is now being prepared in this medium with cultures incubated at 27°-28°C for 6 wk and killed by 0.1 per cent formalin (not by heat) and preserved in phenyl mercuric nitrate (Sokhey *et al* 1950). The mouse-protective dose of this vaccine is 0.004 ml as against 0.2 ml to kill a mouse. The keeping qualities are very good even when stored at 37°C for 18 months. Casein hydrolysate vaccine is highly potent and less toxic as compared to the agar-grown vaccine.

(*b*) *Agar-grown vaccine*: Originally adopted by the German plague commission (1899), Bergess (1930) used whole meat agar, Barreto (1938) preferred glycerol agar, US Army hormone or hormone sulphite agar and Wayson *et al* (1946) blood tryptose beef heart agar. For better growth of envelope substance an incubation temperature of 37°C is now considered preferable than 30°C as used by the US Army laboratory. The mouse-protective dose of these vaccines varied between 0.002 to 0.004 ml. These agar vaccines were standardised by different workers to contain 1000 to 3000 million organisms. Sokhey (1936) used 1500 million per ml.

(*c*) *Y. pseudotuberculosis vaccine*: A vaccine prepared from formalin-killed pseudotuberculosis bacilli (containing 4000 million ml) was introduced by Boye (1933) in Madagascar, its protective value was much less than *Y. pestis* vaccine.

(*d*) *Lipo-vaccine*: This is a modification of Boye's vaccine by suspending the dried Boye's vaccine in an oily recipient. It proved better than Boye's original vaccine.

(*e*) *Sugar vaccine*: The vaccine made out of avirulent strains was originally introduced by Minervin *et al* (1935), but modified later by Korobkova *et al* (1938) who used virulent plague bacilli instead of avirulent organism incubated at 37°C. The organisms were suspended in double volume of saccharose solution of 80 per cent strength and left the mixture at room temperature for 20-25 days to effect sterilisation without heat. Two injections of this vaccine protected 82-100 per cent of white mice and 50-82 per cent guineapigs against a challenge dose of highly virulent plague bacilli.

(*f*) *Precipitated vaccine* (*two types*)

(α) Alcohol-precipitated vaccine: To phenolise saline suspension of virulent plague bacilli grown on blood tryptose beef heart agar for 40 h at 39°C two volumes of 95 per cent ethanol was added and the mixture left at 5°C overnight. Then the mixture was centrifuged and resuspended in saline containing 1/7500 merthiolate to make up the volume of the original phenolised saline standardised suspension.

(β) Alcohol cum-alum precipitated vaccine: To 100 ml of saline the above preparation after overnight incubation at 5°C, 2.7 ml of 10 per cent sodium bicarbonate solution and 2.5 ml of 4 per cent potassium alum solution were added and the mixture was allowed to stand at 5°C for 5 h. Then the supernatant fluid was removed by centrifuging and the sediment resuspended in saline and held again at 5°C for 40 h. The rest of the procedure was the same as that of the alcohol-precipitated type, but this was not found superior to alcohol-precipitated type. Meyer (1950a) obtained better results with formalin-killed and alcohol-precipitated vaccines.

(*g*) *Chemically-prepared extracts of Y. pestis*: Kurauchi and Homma (1931) postulated that the plague bacilli had two antigens *viz.*, (*i*) capsular-the specific immunizing factor; and (*ii*) somatic antigen sharing immunogenic properties with pseudotuberculosis bacillus. These workers extracted the antigen from the plague bacilli by acid acetone which when administered in two doses to guineapigs and rats conferred stronger immunity than ordinary plague vaccine. Meyer (1948) also obtained similar results with various fractions of *Y. pestis* separated by them with the aid of chemical procedures. The author (Seal, 1960b) claimed better protective capacity with the P1/3 sodium sulphate fraction of the precipitate obtained from the supernatant of Haffkine plague vaccine prepared in casein hydrolysate enriched broth. This will be further discussed in Chapter VIII.

(2) *Live Avirulent Vaccine*

Strong as early as 1903 was the first worker to try live avirulent plague bacilli for immunisation of 700 human beings in Manila. But its value could not be ascertained in the absence of plague at that time except that the sera of inoculated persons contained agglutinins against plague bacillus (this test was also unreliable due to auto-agglutination). The next attempt to use live avirulent vaccine was by Girard and Robic, and Otten in 1936 in Madagascar and Java respectively. Following the success achieved by these workers this kind of live vaccine was used between 1941 and 1946 in Argentina, Belgium, Congo, Brazil, French West Africa, Tunisia and Union of South Africa, Pokrovskaya and Kaganova (1945) claimed to have enhanced the defence mechanism of the lung against plague infection through the administration of live avirulent plague vaccine by inhalation but it seems it was not thoroughly tried in human beings.

Girard and Robic (1936) used EV Madagascar strain and Otten (1936)

used Tjiwidej strain at Java. In a later study Girard and Radaody-Ralarosy (1940) recorded that the EV strain possessed invasive power and the protective power was due to some residual virulence. Similarly Tjiwidej strain was also invasive and was toxic to rats when heated at 58°C and injected into these animals. EV strain had also similar properties. On account of these properties and of the problem of maintenance of the immunogenic power workers in India always preferred to use killed Haffkine Plague vaccine, which improved further with the use of casein hydrolysate medium of Seal and Mukherji (1943).

Grasset (1946) used a combination of EV and Tjiwidej strains in concentration of 100 million per ml. Later, however, Tjiwidej strain was replaced by Rowland's K/120 which was found highly immunogenic. In most places of Africa and South America, however, EV strain was used. On the other hand, Korobkova established that guineapigs could be better immunised by a single injection of a mixture of strain 46-S obtained from a virulent plague growth through bacteriophage action and EV strain or by an initial inoculation with 46-S followed one week later by EV inoculation. In brief, live vaccine having its invasive properties was considered to be better immunogenic than killed virulent organism, but the danger of revival of virulence could not be ruled out. Besides the quality of the strain could be maintained only by keeping it at a temperature of 2-4°C and subculturing it not more than once a year. Lyophilisation was considered to be a better method but Girard (1948) found it unsatisfactory. However, recent development in the study of antigens revealed that a protein-polysaccharide complex is the immunogenic moiety and that it can be administered as such with effective protection, thus eliminating the wasteful preparation of liquid vaccine in anticipation of plague outbreak (Seal, 1960b).

Evaluation of Potency of Vaccine

No satisfactory process was available for evaluation of potency of vaccine till 1935. Previously Taylor (1933) considered lower monkeys, guineapigs and rabbits unsuitable and so also wild rats. Sokhey and Maurice (1935, 1937) developed an inbred white mouse very much suitable for potency test, they being fully protected with only 0.03 ml of Haffkine vaccine prepared in casein hydrolysate broth. The method was fully standardised by Sokhey and Maurice (1937) and confirmed by Meyer *et al* (1948).

Passive Immunity to Plague

In the absence of any specific drug till the use of sulphonamides in 1935 (Carman, 1938 and Vine, 1938) antisera produced in horses, buffaloes or rabbits were the mainstay in the treatment of plague and iodine solution intravenously. Goats, sheep, calves, mules and bullocks had also been used for the production of antiplague serum. Out of these antisera produced in horses was the commonest and next to it in rabbits. But the quantity of

antiserum available in rabbits being comparatively small though recommended by Korobkova (1940) and Jawetz and Meyer (1944a). The best antiplague serum was, however, produced at the Haffkine Institute (Sokhey, 1936b). The mouse-protective test dose was 0.05 against 0.30 ml of buffalo serum (Naidu and Mackie, 1931) and 0.50 ml of Pasteur or Lister Institute serum.

The various antigens used for immunisation of horses and other animals were killed plague culture alone or followed by live virulent bacilli, live avirulent bacilii, atoxic filtrates of nucleoproteins, lysates of envelope substance, and plague anatoxin *etc.* At the Haffkine Institute, the immunisation of horses was started with live avirulent culture followed by live virulent culture growth and finally with filtrates of broth culture incubated for 3 wk at 27°C. Meyer (1947) obtained potent antiserum in rabbits and monkeys by immunizing with crystalline 1B plague antigen.

CHAPTER VIII

Immunological Studies of Plague Bacillus and Allied Organisms

Antigenic Structure of *Yersinia pestis* and Allied Organisms

Prior to 1940 the plague workers were facing certain difficulties regarding the physical, chemical and immunological properties of the plague bacillus and related organisms like pseudotuberculosis. The particular difficulty was in regard to the serological behaviour and its differentiation from pseudotuberculosis, as the success of field epidemiology and detection of reservoirs during the interepidemic period largely depended upon this differentiation. Investigations were made by various workers from time to time (Sokhey and Maurice, 1935; Schutze, 1939; Bhatnagar, 1940; Jawetz and Meyer, 1944 b; Seal, 1951 a, b, c, 1954 and 1958; Devignant, 1951 b; Girard, 1953; and Burrows and Bacon, 1956) greatly helped in clarifying the position.

The plague bacilli are now primarily divided into (*a*) virulent and (*b*) avirulent forms. The latter is again subdivided into (*i*) protective and (*ii*) non-protective strains, based on animal tests. The bacillus loses its virulence not only by cultivating it in artificial culture medium but also in the immune rats during the interepidemic periods as observed by the author (Lal and Seal, 1949; Seal, 1958). Serologically, the author (Seal 1951 a) was able to differentiate the virulent from the avirulent non-protective plague and pseudotuberculosis organisms but no differentiation could be made between the virulent and avirulent protective plague organisms except by animal test and quartz ultraviolet spectographic readings of the respective specific proteins. Englesberg *et al* (1954) concluded that virulence was determined by quantitative relation between envelope and toxin production.

Recently, however, Burrows and Bacon (1956) by a special technique discovered two additional antigens called V and W which can differentiate virulent from the non-virulent plague strains being present in the former and absent in the latter. In a further extension of the study Burrows (1959) obtained two additional determinants of virulence namely, P (pigment positive) and Pu (purine producing). The V antigen is related to a property of the related organism by which it resists phagocytosis by mouse polymorphs. He has also studied both freshly isolated and laboratory strains of *Y. pseu-*

dotuberculosis for the same antigen and it was found that the main difference between the two organisms was the absence of F1 in the pseudotuberculosis strains. The other three antigens are the same as those of *Y. pestis* except that in some strains any one of them may be absent. As with *Y. pestis* the virulence can be enhanced with addition of iron in the culture medium. Accordingly the serological antigenic structure of the plague and pseudotuberculosis organisms stands as follows:

Organisms		*Antigens*
Y. pestis virulent	F1+*VW+P+Pu	Common rough somatic antigen
Y. pestis avirulent protective	F1+VW−, P+Or−, Pu+	Common rough somatic antigen
Y. pestis avirulent nonprotective	F1−, VW−, P−Or+, Pu−	Common rough somatic antigen
X. pseudotuberculosis virulent (fresh)	F1−VW+P+, Pu+	Flagellar smooth and somatic smooth (type and group specified)
X. pseudotuberculosis virulent (laboratory)	F1−VW−, P+, Pu+	Common rough smooth

*F1 of Baker *et al* is the same as A of Seal and relation to the envelope substance.

Devignat (1951 b, 1958), on the other hand, placed all the world strains into three noso-graphical varieties, based on their biochemical behaviour and on clinico-pathological effects (virulence). These are:

i) var *orientalis*—This strain does not ferment glycerine, transforms nitrates into nitrites and produces nitric acid in broth without nitrates. It causes late septicaemia in mice. It is found in India, South China, Morocco, Madagascar, Indonesia, S. Africa and USA. This type corresponds to Berlin and Borzenkov's (1938) *oceanic* strains.

ii) var *mediavalis*—It ferments glycerine, transforms the nitrates but does not produce nitric acid; seems to be pneumotropic to some extent and is found in South East Russia and Kurdistan.

iii) var *antiqua* It ferments glycerine, transforms the nitrates but does not produce nitric acid. It shows a tendency to provoke septicaemia and is found in the North East Asia, North China and Belgian Congo (Central Africa).

Pollitzer (1954) however, suggested further work on Devignat's classification on the basis of Girard's earlier work (Girard, 1940; 1950 b).

Isolation, Fractionation and Properties of Specific Soluble Proteins of *Y. pestis* and Allied Organisms

In the selection of plague strains for the preparation of antiplague vaccine the divergent opinions were expressed by different workers from time to time. The existing knowledge on the antigenic structure was not considered dependable for the purpose. The solution of this problem thus involved the necessity of determining the chemical antigenic fractions of the organism

and their relation to the different forms of *Y. pestis*, both virulent and avirulent, which have been used for the preparation of vaccine under different conditions of maintenance and growth.

Several workers (Lustig and Galleoti, 1897; Balfour-Stewart, 1900; Schutze, 1932; Sokhey and Maurice, 1935; Sokhey, 1939 a; and Baker *et al*, 1947) had earlier worked on the identification and isolation of the protective antigen of plague bacillus as referred by the author (Seal and Mukherjee, 1950). These workers concluded that the protective antigen was either protein or nucleoprotein in nature and was supposed to be loosely attached to the organism. Since this specific substance of plague bacillus was protein in nature these workers had to face difficulties in isolating this substance by using liquid media containing alien protein. Shrivastava (1939) attempted to overcome this difficulty by introducing gelatin hydrolysate medium as prepared by Rao (1939) but the medium was not completely protein free. The present author (Seal and Mukherjee, 1950) found his protein free casein hydrolysate broth very handy to overcome this difficulty and to isolate the specific protein from the supernatant of the culture made in this medium.

Baker and his co-workers (1947) isolated water soluble and water-insoluble fractions from the saline suspension of the bacilli grown on nutrient agar for 3 days at 37°C. Since these media did not prove very efficient for the growth of virulent plague bacilli, an attempt was also made to isolate and study the protein substance from the water soluble extracts of the organism grown on casein hydrolysate agar, a new improved solid medium described earlier.

Methods of Isolation of the Fractions

i) From Casein Hydrolysate Broth

In a preliminary experiment with the culture filtrate of virulent plague bacillus the yield of the crude protein was highest by precipitation with Na_2SO_4 as compared to acetic acid and with 3 weeks period of incubation at 28°C than with shorter period of incubation. For each strain five fractions were isolated at different concentrations of sodium sulphate namely, one-third, one half, half minus one third and full minus half ($P\frac{1}{3}$, $P\frac{1}{2}$, $P\frac{1}{2}$-$\frac{1}{3}$, PI-$\frac{1}{2}$) and the residue (P standing for the protein precipitate and fraction represents the degree of saturation with Na_2SO_4) at a constant temperature of 37°C. Each fraction was dialysed in cold water for 7-11 days until sulphate free and then dried over P_2O_5 in vacuo, to a constant weight. The percentage of nitrogen recovery was estimated by microkjeldahl technique. The fraction $P\frac{1}{3}$ was most highly active giving a precipitation titre of 2,000,000 as against 200,000 by $\frac{1}{2}$-$\frac{1}{3}$ fraction, the respective quantitative equivalent being 7 and 32 mg against 1 ml of a potent antiplague horse serum. Only a small amount of serologically inactive precipitate was obtained raising the concentration of Na_2SO_4 from $\frac{1}{2}$ to full saturation. Thus the

active protein substance in the supernatant of the Haffkine plague vaccine could be precipitated by half saturation with $Na_2 SO_4$ the most active part of it being contained in the precipitate obtained by one third saturation of the salt. This portion was designated as antigen A and the P $\frac{1}{2}$-$\frac{1}{3}$ as antigen B.

ii) From Culture on Casein Hydrolysate Agar

In a preliminary experiment a virulent plague strain (137/L) was grown for 72 h at 37°C on casein hydrolysate agar in 24 Roux bottles each containing 150 ml of agar. The growth was washed in distilled water and after testing for purity the washings of 7, 6, 6 and 5 bottles were separately pooled in 4 litre sterile Haffkine flask and the volume made up to 2 litres in each. The suspensions were then kept at 37°C for 3, 7, 10 and 14 days respectively with occasional shaking to help dissolution of the soluble protein of the suspended organism the respective bacterial contents being 1000, 800,800 and 600 million per ml. At the end of the respective periods the emulsions were made bacteria free by Keiselgular and Seitz filtration and then precipitated with $Na_2 SO_4$ at P $\frac{1}{3}$, P $\frac{1}{2}$, P $\frac{1}{2}$-$\frac{1}{3}$, P 1-$\frac{1}{2}$ fractions and dialysed and dried to a constant weight as in case of protein fractions isolated from casein hydrolysate broth.

The results showed progressive autolysis of the organism up to 14 days and as high as 86 per cent of the solids and 69.3 per cent of the nitrogen content of the bacterial bodies went into solution. P $\frac{1}{2}$-$\frac{1}{3}$ fraction constituted 10 per cent by weight of P$\frac{1}{2}$ fraction except in case of 2 days extraction in which 5-10 per cent of the precipitate did not go into solution during purification. The nitrogen recovery was highest with 600 million organism per ml. With higher concentration of organism it varied between 55 and 58 per cent irrespective of the period of extraction. The highest yield of protein 0.2 mg per 1000 million of organism was then obtained with 600 million organisms per ml. A subsequent experiment with 500 million organisms per ml showed that ninety per cent of the bacterial body went into solution in 14 days. The precipitation titre against antiplague sera was 1,280,000 in case of P$\frac{1}{3}$ fraction and 120,000 in case of P$\frac{1}{2}$-$\frac{1}{3}$ fraction; with 600 million organisms per ml the emulsion became sterile in 12 days at 37°C. The above studies were then extended to avirulent protective plague strains 53/Hav, avirulent nonprotective plague strains 120/5Hav and TRU and pseudotuberculosis strains PR/I and PR/IV.

Certain Chemical, Physical and Serological Properties of the Isolated Protein Fractions

The P$\frac{1}{3}$, P$\frac{1}{2}$-$\frac{1}{3}$ and P$\frac{1}{2}$ fractions of the two virulent plague, one avirulent protective plague, two avirulent non-protective plague and two *P. pseudotuberculosis* strains as mentioned earlier and isolated either from CH broth filtrates or from the water soluble extracts of the organism grown on casein hydrolysate agar were studied. To distinguish the latter from the former the

symbol L/auto P was used.

i) Physical Properties

(*a*) *Physical appearance and solubility*—All the protein fractions were isolated as amorphous substance which dried into form of scales. Those obtained from the broth filtrate had a brownish tinge (obtained from the colour of the broth) and formed solute with a faint colour in water or salt solution (0.6 to 1%). The samples obtained from the extracts of the agar grown organism were greyish and lighter in weight and formed whitish or colourless solution in water and salt solution. The $P\frac{1}{2}$-$\frac{1}{3}$ fractions were generally lighter than the other fractions in both weight and colour.

The solubility was generally low at room temperature (30°-35°C). It improved with *p*H 7.8-8.0 and complete solution was obtained in N/80 NaOH. The solubility in distilled water was rarely more than 1 : 500, the highest 1 : 200 being recorded in case of L/auto $P\frac{1}{3}$. Thus the solubility could not be used as a guide of the purity of the substance.

(*b*) *Specific rotation and absorption spectra of the protein solutions*—Attempts to differentiate the protein fractions of different organisms by means of polarometric reading failed due to the samples being of heavy colloidal nature and faintly tinged. The optical activity of these solutions under the influence of alkali (racemization) was not tried.

(*c*) *Spectrographic readings*—The protein fractions were dissolved in N/80 NaOH to make 0.04 per cent clear solution (stronger solution was not suitable). The absorption curves were obtained by plotting the extinction coefficients against wave lengths the absorption range of all the protein solutions lay near the ultraviolet region between 230 and 430 mμ (Fig. 32). In the case of protein fraction L3 $P\frac{1}{3}$ and L/auto $P\frac{1}{3}$ isolated from virulent *Y. pestis* had a moderate bend in the absorption curve between 268 and 305 mμ which was not seen with the other preparations. This might be taken as evidence of difference between virulent and avirulent plague strains.

(*ii*) *Certain Chemical Properties of the Protein Fractions*

The nitrogen values of $P\frac{1}{3}$ fractions of virulent and avirulent protective plague strains varied between 15.1 and 15.8 per cent against 14.9 to 15.6 per cent of $P\frac{1}{2}$ fractions. A higher reading was, however, obtained by autolysate fractions indicating these to be purer than these obtained from CH broth. Consistently lower values were obtained by fractions isolated from avirulent non-protective plague and pseudotuberculosis strains.

The fractions were also tested for P, S, tryptophane and tyrosine and for Molisch and biuret reaction. The results indicate that all the proteins tested were sulphur containing phosphoproteins. The hydrolysate (with H_2SO_4) gave positive Molisch, biuret, PO_4 and Xanthoprotein tests. Tests for tryptophane and tyrosine showed that the $P\frac{1}{2}$ fractions obtained from the protective plague strains from CH broth contained both these amino-acids and those from non-protective plague and pseudotuberculosis strains little

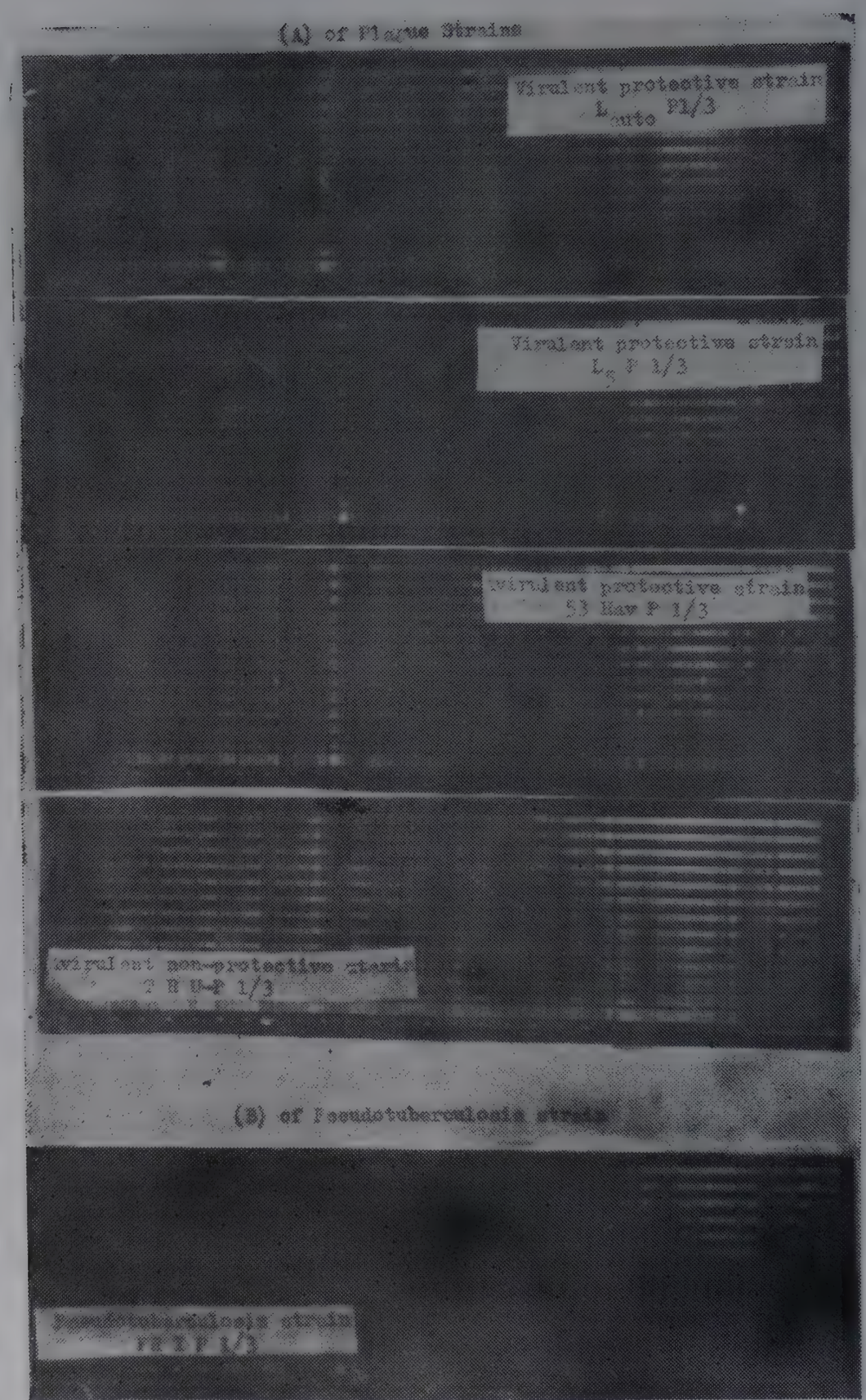

Fig. 32. Spectrograph of protein fractions of plague and pseudotuberculosis strains.

or none, whereas those obtained from the water soluble extracts did not contain any of them. Since tryptophane was virtually absent in the CH broth its presence in the fraction apparently indicate that the plague bacilli could synthesize this amino acid during their growth in CH broth and absence of this aminoacid in the autolysate may mean that it is a constituent of the inner core of the organism and not of the surface which Schutze (1932) termed as 'Envelope substance'.

(iii) *Serological Properties*

Antisera were produced in rabbits against the protein fractions as well as the whole organism. Two rabbits were immunised for each fraction by 4 doses *viz.*, 1, 1.5, 2 and 4 mg in physiological salt solution at biweekly intervals and then bled from the heart 1 week after the lastin jection. For producing antisera against virulent plague bacilli rabbits were given weekly subcutaneous injection of live organism beginning with 1 ml of a 5-millionth to a millionth-dilution of live suspension containing 100 million organisms, increasing the dose tenfold in the successive injections until the animal received 1 ml of the suspension and doubling the dose at each successive interval. The first bleeding was done 1 week after the animal had received 1 ml of the suspension intravenously (the total number of doses being 10-12). Almost the same procedure was for other organisms. The results of homologous and cross precipitation reactions of protein fractions against various anti-protein and anti-plague and anti-pseudotuberculosis sera are given in Tables 29 and 30.

The results of agglutination and cross agglutination titres of the antiprotein sera against various plague and allied organisms are given in Table 31.

It is apparent from the results given in Tables 29-31 that L/auto $P\frac{1}{3}$ antiserum is highly specific so far as the serological reactions are concerned. This fraction is not precipitated by antisera produced against either the protein fractions or the whole organism of the pseudotuberculosis, avirulent non-protective plague and boiled virulent plague strains. Also, conversely an antiserum against the above fraction neither agglutinates organisms other than the protective plague strains nor precipitative protein fractions obtained from them. L/auto $P\frac{1}{2}$-$\frac{1}{3}$ fraction isolated only as a small fraction of the water soluble extracts, is not sharply differentiated from L/auto $P\frac{1}{3}$ as also seen from the results of the ring precipitation test. The results also show that when the antisera against either virulent or avirulent protective strains are absorbed with avirulent non-protective plague or pseudotuberculosis organisms the residual antisera react with the above protein fraction. Thus L/auto $P\frac{1}{3}$ fraction may be suitably utilised for differentiating protective from the non-protective plague strains but it still fails to differentiate virulent from avirulent plague strains.

L/auto $P\frac{1}{2}$ is antigenically broader than L/auto $P\frac{1}{3}$ and cross reacted with 120/Hav antiserum indicating certain admixture with $P\frac{1}{2}$-$\frac{1}{3}$ fraction. Thus the protein fraction obtained by 'one-third' saturation of the filtrate of the

Table 29. Homologous and cross tube reactions of protein fractions against various antiprotein, antiplague and anti-pseudotuberculosis sera

Antisera protein fraction	Total precipitate titres × (1000)									
	L auto $P_{\frac{1}{3}}$ 1 : 10	L_5 $P_{\frac{1}{3}}$ 1 : 10	L_5 $P_{\frac{1}{2}\text{-}\frac{1}{3}}$ 1 : 10	L_5 $P_{\frac{1}{2}}$ 1 : 10	L_3 $P_{\frac{1}{3}}$ 1 : 10	337/L 1 : 10	53/Hav 1 : 10	120/ 5Hav 1 : 10	PR/I 1 : 10	PR/IV 1 : 10
L auto $P_{\frac{1}{3}}$	500	500	0	50	50	1000	1000	0	0	0
L auto $P_{\frac{1}{2}\text{-}\frac{1}{3}}$	20	20	0	±	0	50	50	0	0	0
L_5 $P_{\frac{1}{3}}$	250	250	0	100	100	1000	500	50	±	±
L_5 $P_{\frac{1}{2}\text{-}\frac{1}{3}}$	±	20	50	20	—	100	50	20	50	50
P_5 $P_{\frac{1}{2}}$	100	100	20	100	50	500	250	50	20	24
L_3 $P_{\frac{1}{3}}$	20	50	0	50 (f)	250	250	250	20	0	0
53/Hav $P_{\frac{1}{3}}$	100	100	±	20	100	1000	1000	50	20	20
53/Hav $P_{\frac{1}{2}\text{-}\frac{1}{3}}$	—	—	—	—	—	—	—	—	—	—
120/5Hav $P_{\frac{1}{3}}$	0	0	±	20	20	100	50	250	50	50
120/5Hav $P_{\frac{1}{2}\text{-}\frac{1}{3}}$	—	—	—	—	—	—	—	—	—	—
TRU $P_{\frac{1}{3}}$	—	—	—	—	—	—	—	—	—	—
PR/I $P_{\frac{1}{3}}$	0	C	C	C	C	C	C	20	100	50
PR/IV $P_{\frac{1}{2}}$	0	C	C	C	C	C	C	20	50	250

f = faint; — = not done; 0 = < 20,000; C = cloudy; ± = doubtful.

Table 30. Ring precipitation reactions of protein fractions against various antiprotein, antiplague and anti-pseudotuberculosis sera

Antisera protein fraction	Ring precipitation									
	53/Hav $P_{\frac{1}{3}}$ 1 : 10	53/Hav $P_{\frac{1}{2}\text{-}\frac{1}{3}}$ Undil.	120/Hav $P_{\frac{1}{3}}$ Undil.	120/Hav $P_{\frac{1}{2}\text{-}\frac{1}{3}}$ Undil.	TRU $P_{\frac{1}{3}}$ Undil.	PR/I $P_{\frac{1}{3}}$ Undil.	PR/I $P_{\frac{1}{2}\text{-}\frac{1}{3}}$ Undil.	PR/IV $P_{\frac{1}{3}}$ Undil.	PR/IV $P_{\frac{1}{2}\text{-}\frac{1}{3}}$	337/L '0' Undil.
L auto $P_{\frac{1}{3}}$	+	0	0	0	0	0	0	0	0	0
L auto $P_{\frac{1}{2}\text{-}\frac{1}{3}}$	±	−	±	±	±	0	0	0	0	0
L_5 $P_{\frac{1}{3}}$	+	0	0	0	0	0	0	0	0	0
L_5 $P_{\frac{1}{2}\text{-}\frac{1}{3}}$	±	+	0	0	+	+ (f)	0	+ (f)	0	+
L_5 $P_{\frac{1}{2}}$	−	−	−	−	−	−	−	−	−	−
L_3 $P_{\frac{1}{3}}$	+ (f)	+ (f)	+ (f)	0	0	±	0	±	0	±
53/Hav $P_{\frac{1}{3}}$	+	0	+ (f)	0	+ (f)	±	0	+ (f)	0	0 (53/Hav $P_{\frac{1}{2}}$ = +)
53/Hav $P_{\frac{1}{2}\text{-}\frac{1}{3}}$	0	+	+	0	0	+	0	+	±	±
120/5Hav $P_{\frac{1}{3}}$	±	+	+	0	+	++	+	++	+	+
120/5Hav $P_{\frac{1}{2}\text{-}\frac{1}{3}}$	0	0	±	+	±	++	0	±	±	±
TRU $P_{\frac{1}{3}}$	±	+	+	0	+	++	+ (f)	+	+	± (TRU $P_{\frac{1}{2}}$ = +)
PR/I $P_{\frac{1}{3}}$	±	+	+	+	+	++	+	++	+	+
PR/IV $P_{\frac{1}{2}}$	0	+	+	+	+	++	+	++	+	+

f= faint; − = not done; 0 = negative; ± = doubtful.

Table 31. Agglutination and cross agglutination titres of the antiprotein sera against various plague and allied organisms (min. dil. 1 : 25)

Antiprotein sera	Organism (suspension)					
	337/L	53 H/av.	120 H/av.	TRU	PR/I	PR/IV
L/auto $P_{\frac{1}{3}}$	400	200	0	0	0	0
L_5 $P_{\frac{1}{3}}$	200	200	25	0	—	—
L_5 $P_{\frac{1}{2}\text{-}\frac{1}{3}}$	0	0	50	25	25	25
L_5 $P_{\frac{1}{2}}$	100	100	25	25	25	25
L_3 $P_{\frac{1}{3}}$	100	200	50	50	50	50
L_3 $P_{\frac{1}{2}}$	100	100	200	200	100	100
53/Hav $P_{\frac{1}{3}}$	100	100	25 (f)	0	0	0
53/Hav $P_{\frac{1}{2}\text{-}\frac{1}{3}}$	0	0	100	25	25	25
120/5Hav $P_{\frac{1}{3}}$	0	0	100	100	25	25
120/5Hav $P_{\frac{1}{2}\text{-}\frac{1}{3}}$	0	0	100	±	25	50
TRU $P_{\frac{1}{3}}$	0	0	0	50	25	50
PR/I $P_{\frac{1}{3}}$	0	0	25	50	50	25
PR/I $P_{\frac{1}{2}\text{-}\frac{1}{3}}$	0	0	25	50	50	50
PR/IV $P_{\frac{1}{3}}$	0	0	25	±	25	50
PR/IV $P_{\frac{1}{2}\text{-}\frac{1}{3}}$	0	0	50	±	50	50

supernatant of Haffkine plague vaccine or of the water extract contains the antigen which is specific for the virulent and protective plague strains. L_5 P ($\frac{1}{2}$-$\frac{1}{3}$) fraction reacts with antisera against 53/Hav P ($\frac{1}{2}$-$\frac{1}{3}$), TRU $P_{\frac{1}{2}}$, PR/I $P_{\frac{1}{3}}$ and PR/IV $P_{\frac{1}{3}}$ protein fractions as well as against 337/L, 53/Hav, 120/5Hav, PR/I and PR/IV strains and 337/L boiled organisms. The antisera against the fraction itself, does not agglutinate 337/L and 53/Hav but agglutinate 120/Hav, TRU, PR/I and PR/IV strains. 53/Hav P ($\frac{1}{2}$-$\frac{1}{3}$) also behaves in the same way as L_5 P ($\frac{1}{2}$-$\frac{1}{3}$). These results indicate that this fraction may be acting as the common link amongst the virulent and avirulent protective and avirulent non-protective plague and pseudotuberculosis organisms. It is also related to the somatic 'O' antigen of the virulent plague strain. Thus the relationship of the plague to the pseudotuberculosis strain is through the somatic antigen represented by P ($\frac{1}{2}$-$\frac{1}{3}$) fraction of broth filtrate of virulent plague bacillus. This $P_{\frac{1}{3}}$ fraction of virulent plague strains may be designated as antigen A and P ($\frac{1}{2}$-$\frac{1}{3}$) fraction as antigen B, linking virulent and avirulent protective plague strains with avirulent non-protective and pseudotuberculosis strains.

The antisera against PR/I and PR/IV do not agglutinate 337/L and 53/Hav strains but agglutinate 120/Hav, TRU, PR/I and PR/IV strains and react as mentioned above, with L_5 ($P_{\frac{1}{2}\text{-}\frac{1}{3}}$), 50/Hav P ($\frac{1}{2}$-$\frac{1}{3}$) or TRU $P_{\frac{1}{3}}$ protein fractions *i.e.*, antigen B). The pseudotuberculosis strains are also agglutinated and their protein fractions precipitated by 337/L 'O' antiserum, thus indicating that these two strains have, in their somatic constitution, an antigen common with the virulent plague organism and their dissociants through the

specific soluble protein fraction identified as antigen B.

Antigenic Classification of Strains

On the basis of the above two antigens the plague strains could be classified into three groups *viz.*, (1) those containing both A and B antigens *e.g.*, all virulent and relatively avirulent plague strains (2) those containing only a small amount of residual antigen A and full antigen B as shown by a partial cross-reaction of such organisms with the antibody against antigen A and high reaction with anti-pseudotuberculosis serum [which do not react at all with the strains of group (1)] and (3) those which contained only antigen B *e.g.*, avirulent non-protective plague and all pseudotuberculosis strains.

The above studies, however, do not permit a clear cut differentiation between virulent and avirulent protective plague strains. A difference has, however, been noted in the extinction coefficient curve of $P\frac{1}{3}$ fraction (antigen A) of virulent plague strains between 268 and 305 $m\mu$ wave length in the ultraviolet region of the spectrograph. Another difference noted was while this antigen constituted 90-95 per cent of $P\frac{1}{3}$ fraction of the virulent plague strains, the same for the avirulent protective *Y. pestis* constituted only 55 per cent. Thus the isolation of the specific soluble protein fractions facilitated the following studies:

a) differentiation between the protective and non-protective plague strains;

b) quick and ready identification of *Y. pestis* and the differentiation from *P. pseudotuberculosis* in rodents during field investigations;

c) development of *in vitro* method of standardisation of antiplague serum by quantitative precipitation technique of Heidelberger as an alternative method to the time-consuming mouse-protection test;

d) rough and ready method of estimating protective value of antiserum from horses or rabbit during the process of immunisation by Dean and Webb's flocculation technique;

e) use of purified protein substance as an immunising agent in place of Haffkine antiplague vaccine which cannot be preserved long;

f) development of qualitative and quantitative precipitation test to evaluate the antigenic power of antiplague vaccine in animals or in human beings; and

g) development of complement fixation test as a diagnostic procedure in plague infection or to assess the immunity status of persons immunised against plague infection.

This study further indicated that there was a change of the antigenic structure of the plague strains by frequent subculture in the laboratory.

Isolation of Active Polysaccharide from Plague Organisms

In the study of the chemical antigenic structure it was of interest to know if any polysaccharide or carbohydrate moiety was involved in the

specific soluble protein antigen. In case of *V. cholera*, it was the polysaccharide moiety of the polysaccharide protein complex antigen, that gave the specificity. However, an attempt to isolate an active polysaccharide from the supernatant of the Haffkine plague vaccine was made by the earlier workers (Shrivastava, 1939) but the yield was too small for study. The new protein free casein hydrolysate broth offered another opportunity to reopen the investigation. Attempts were, therefore, made to isolate this substance from three sources by (1) supernatant of antiplague vaccine prepared in casein hydrolysate broth, (2) the bacterial debris of the same vaccine and (3) the specific soluble protein of plague bacillus.

A polysaccharide yielding osazone resembling that of arabinose with melting point of 166°-168°C was isolated from the supernatant of the Haffkine plague vaccine as well as from the specific soluble protein and to a small extent from the bacterial debris of both virulent and avirulent protective plague strains. It was absent in the non-protective plague strains and pseudotuberculosis organism (Seal, 1951b). This polysaccharide protein complex reacted with antiserum raised against either virulent or avirulent protective plague strains and this property was retained even after absorption of those antisera with non-protective avirulent plague and pseudotuberculosis organisms, indicating that the immunising substance is related to the specific soluble protein or antigen A of plague bacillus and not to the somatic antigen B. It was, therefore, concluded that the protective substance of plague bacillus is a polysaccharide, protein complex or a nucleoprotein derivative.

CHAPTER IX

Serological Methods for the Diagnosis of Plague

The various processes utilised for sero-diagnosis of plague are agglutination, haemagglutination, precipitation, complement-fixation and flocculation.

Agglutination

Agglutination test with plague organism was handicapped due to its instability in salt solution, causing auto-agglutination. In 1939, Wats *et al* devised a complicated process in the preparation of suspension of the bacilli by shaking with sterile glass beads to break up the coarse particles and placing it in a refrigerator overnight. The homogeneous supernatant was centrifuged and the deposit washed in saline twice and resuspending it in normal saline containing 0.25 per cent phenol and 0.05 per cent formalin. Devignat (1942) obtained a fairly homogeneous growth of *Y. pestis* by bubbling air through the broth culture and after stabilising the growth by adding formalin and then using this suspension for agglutination reaction. This author overcame the difficulty by using live culture grown on casein hydrolysate agar. The standard antiserum was raised in rabbits by using the same type of suspension, the titre rising as high as 1:2560. Baker *et al* (1947) obtained immune serum by raising it in rabbit against 1A and 1B antigens while the author (Seal, 1953) raised the antiserum in rabbits by injecting water-extractable protein fraction of plague bacillus and for mass serological diagnosis of the immunity in rats in field studies he devised a gel-precipitation test (Seal 1972 b).

Types of Agglutination

Two distinct types of agglutination can be discerned depending upon the nature of the plague strains (Wats *et al* 1939) using the same antiserum namely, (i) floccular; and (ii) granular. Bhatnagar (1940), however, ascribed them to the presence of two antibodies in plague immune serum. He differentiated the floccular or wooly type as due to envelope substance and granular or gritty small flakes due to the absence of envelope substance in *Y. pseudotuberculosis* or avirulent non-protective plague strains. He further stated that pseudotuberculosis antiserum did not agglutinate virulent plague strains

and plague antiserum absorbed with pseudotuberculosis strains no longer agglutinated pseudotuberculosis strains. Seal (1951 a) made a detailed study with antisera raised against virulent *Y. pestis*, *Y. pestis* boiled for 1/2 h, *Y. pseudotuberculosis*, water soluble protein extract of *Y. pestis* and with mutually absorbed sera. He clearly showed that not only the differences between plague and pseudotuberculosis strains could be discerned, the inter-strain differences among plague strains could also be made out.

For quick identification of plague strains slide agglutination with specific plague antiserum is good enough. This may be followed by a regular tube-agglutination test for confirmation.

Improved Serological Methods for Diagnosis of Plague and Assessment of Immunity Status

Absorption and cross-absorption of agglutinins: Isolation of specific soluble proteins with identification of chemical antigenic structure of different types of plague and pseudotuberculosis strains has considerably enhanced our knowledge in dealing with the plague organism but it involves high technical skill to isolate the antigen in pure form. To devise a simple method serological studies based on agglutination, precipitation and complement-fixation reactions were carried out with considerable suceess. The three main reasons for which the earlier attempts failed are: (i) the plague strains formed unstable suspension in normal saline; (ii) an antiserum against virulent plague organism agglutinated both plague and pseudotuberculosis strains and the latter at a much higher titre than that of homologous strains; and (iii) the agglutination titre of the same organism incubated at different temperature differed against the same antiserum. The above mentioned and other minor abnormalities involved were finally resolved.

Though the same two antigens as originally proposed by Schutze (1932) were identified by Bhatnagar (1940) but no distinct serological classification of the strains was suggested nor any indication was given as to what organism should be used for the preparation of vaccine. Further work on this line was therefore indicated, which also involved the associated studies on colony characteristics, for the purpose of correlating these with other serological and biological (virulence *etc.*) behaviours. The serological methods presented below eliminated the above difficulties. It was notable that the preparation of antigen suspension, antisera and the actual technique of the test were all of equal importance.

Preparation of antigen suspension: It was experienced (Linton and Seal, 1935; Seal, 1935) that better results could be obtained by using young live suspensions without any preservative or antiseptics which led the authors to try along with other kinds of antigen, living suspension of fairly young culture (24 h) without subjecting it to any possible mutilation with chemicals, heat or antiseptics. 48 h growth was also found equally good but better emulsion was obtained by passing the emulsion once through a pellet of sterile cotton, particularly in case of non-protective plague and pseudo-

tuberculosis strains. The various antigens used in the preliminary experiment were: (1) living, (2) boiled for 15 min, (3) killed at 55° for 1/2 h from each of the cultures grown on (a) acid-digest agar, (b) 10 per cent serum agar and (c) 5 per cent rabbit blood agar. The suspension was made in normal salt solution, half normal saline and distilled water. Normal serum controls were also kept along with that of saline or distilled water.

Out of all suspensions the live suspension made out of 5 per cent rabbit blood agar without any antiseptic or chemical was found to be most stable and reliable. The technique of preparing this suspension was as follows: The culture was withdrawn by means of a loop and suspension made in the normal salt solution at *p*H 7.2 in a test tube and shaken lightly till the opacity equivalent to Brown's opacity No. 2 for plague and No. 3 for pseudotuberculosis was matched. These live suspensions were then ready for use immediately and auto-agglutination was entirely avoided. The organisms were incubated at 37°C in the culture tube except the non-protective E.V. strains which gave better suspension from culture at 28°C, but less specific. It should however be mentioned that strains of all categories do not always make an ideal suspension, *e.g.*, TRU, PM and pseudotuberculosis strains usually form rugose colonies even on blood agar. This can be largely overcome by using cultures of 18-24 h only.

Besides the live suspensions, mentioned above, other antigens used for agglutination and absorption of agglutinins mainly to help in the analysis of the antigenic structure of the bacillus were: (a) heat-killed organisms with or without preservative; (b) alcohol-treated organisms; (c) boiled organisms as 'o' antigen; and (d) the specific soluble protein. These were made according to the standard methods except the specific soluble protein fractions, $P\frac{1}{3}$, $P(\frac{1}{2}\text{-}\frac{1}{8})$ and $P\frac{1}{2}$ from various strains grown from 3 wk on casein hydrolysate broth at 28°C, according to the method developed by the author (Seal, 1951 a).

Agglutinating serum: In the production of agglutinating serum in rabbits against the whole organisms, live suspension prepared as above was used initially by treating with formalin followed by live organisms and careful dosing, irrespective of the type of organism-virulent or avirulent. Antiserum against specific soluble protein was also used for agglutination test. These antisera were either produced by injecting purified protein antigen A of Seal (corresponding to 1A of Baker *et al*) or by the water extracted protein solution of virulent plague strain obtained by suspending the organism in distilled water at a concentration of 500 million organisms per ml and incubating it for 14 days and then making it free from bacterial debries by filtration.

Technique of agglutination test: To various dilutions of antisera placed in uniform bore Felix tubes equal amount of suspension is added and each tube gently shaken. The mixture is incubated at 37°C overnight and the readings taken next morning (a preliminary reading may be taken 2 h after incubation). The highest dilution at which the agglutination is visible to the naked eye is recorded provided the normal serum and normal saline controls

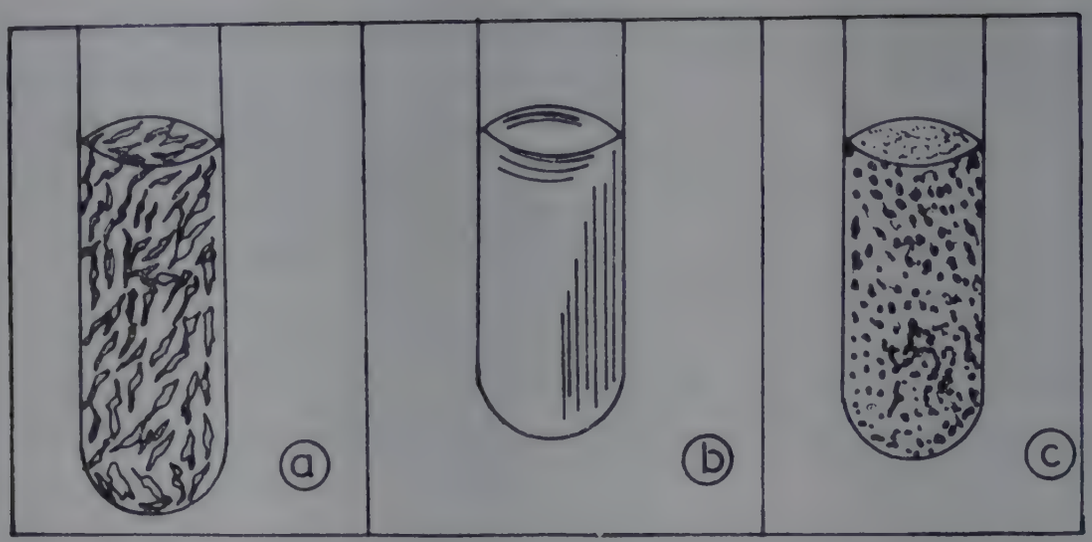

Fig. 33. Agglutination of *Y. pestis*: (a) wooly flocculation type of virulent *Y. pestis*; (b) Negative agglutination of control virulent *Y. pestis*; (c) granular somatic type of agglutination of avirulent non-protective *Y. pestis*.

are negative.

The agglutination reaction is of two types *viz*., (i) floccular or woolly; and (2) granular (Fig. 33). The former is related to the surface and protective antigen giving comparatively low titre agglutination and the latter is related to the somatic antigen which generally gives high titre agglutination.

The results of direct and series of cross agglutination with absorbed and unabsorbed sera (Seal, 1951 a) more or less confirmed the preliminary observation made in connection with the results of direct and cross-agglutination tests. No distinction could be made between the virulent and avirulent protective plague strains but difference was obvious between the protective and non-protective group and pseudotuberculosis strains. While these were capable of absorbing the somatic antibody they were unable to absorb the antibody against the surface antigen (corresponding to envelope antigen of Schutze, 1932) except as residual surface antigen in some of the non-protective strains like PM and TjR.

Specificity of agglutination test: By using three antisera, namely (i) against fraction A or water soluble protein of virulent *Y. pestis*; (ii) against *Y. pestis* suspension boiled for ½ h; and (iii) *Y. pseudotuberculosis* antiserum absorbed with *Y. pestis* live or boiled, a serological distinction between *Y. pseudotuberculosis* and virulent and avirulent protective plague strains could be made. The last antiserum being useful in distinguishing pseudotuberculosis strains from avirulent non-protective plague strains which are found in chronic or mitigated plague in rats and as such need to be differentiated from the former. The serologic relationship between the above organisms is shown in Table 32 (Pollitzer, 1954).

For diagnosis of suspected case of plague or retrospective diagnosis of human plague slide agglutination test might give quick result but the tube agglutination test as described above is the best. The author had the opportunity of testing 26 bacteriologically positive cases, 55 clinically suspected cases and 55 nonplague cases in 1949 in connection with the Calcutta epide-

Table 32. Serological relationship between *Y. pestis* and *Y. pseudotuberculosis*

Antisera produced against	*Y. pestis*		*Y. pseudo-tuberculosis*
	Virulent and avirulent protective	Avirulent non-protective	
Virulent *Y. pestis*	+	+	+
Virulent *Y. pestis* absorbed with *P. pseudotuberculosis*	+	0	0
Y. pestis boiled for 30 min	0	+	+
Y. pseudotuberculosis	0	+	+
Water extractable protein of *Y. pestis* (virulent)	+	0	0
Y. pseudotuberculosis absorbed with *Y. pestis* boiled	0	0	+

Table 33. Results of agglutination test in bacteriologically positive and suspected human cases of plague in 1949

Nature of case	No. tested	Date of collection	Date of examination	Positive	Per cent positive	Agglutination titre
Bacteriologically positive	26	7-40 days after onset	26-130 days after onset	23 (3doubtful)	88.4	1/10 to 1/200
Suspected case bacteriologically negative or not done	56	6-36 days after onset	24-134 days after onset	5	9.0	1/10 to 1/100
Non-plague case	55	—	—	2 (both inoculated)	3.6	1/28 to 1/50

A minimum titre of 1/20 was taken to be diagnostic of plague infection.

mic. The results are given in Table 33. It will be seen that the agglutination test gives a fairly reliable result. It may be mentioned that this was obtained in spite of sulphonamide and streptomycin treatment. No positive serum was tested earlier than 7 days after the onset of symptoms.

According to the results summarised in Tables 32 and 33 the use of antiautolysate plague protein serum or, in its absence antiplague (virulent) serum properly absorbed with *Y. pseudotuberculosis*, can be used as the

plague specific serum for identification of *Y. pestis* without any cross-reaction with *Y. pseudotuberculosis*. Furthermore, for identifying avirulent non-protective plague strains as obtained from laboratory subculture and those which may exist under natural condition two antisera namely (i) Anti-plague (virulent) or anti-pseudo-tuberculosis serum; and (ii) antipseudotuberculosis serum absorbed with virulent live or boiled plague organism may be used. The avirulent non-protective strain will be agglutinated by No. 1 but not by No. 2 antiserum while the latter will agglutinate only the pseudotuberculosis strains.

Antigenic Relationship between Different Strains of *Y. pseudotuberculosis* and *P. avisepticus*

The antigenic structure of *Y. pseudotuberculosis* was studied by Schutze, Kauffman and Bhatnagar. The results of direct and cross agglutination to indicate antigenic relationship between pseudotuberculosis strains themselves and *Y. aviseptica* are shown in Table 34.

Haemagglutination: Haemagglutination may be performed as an indirect method of agglutination by absorbing the organism to erythrocytes of human, sheep or guineapig. In the opinion of Amies (1951) this test was unlikely to be of much value as a diagnostic procedure because of low development of immunity in plague but perhaps it might be useful in retrospective diagnosis of plague to confirm clinical diagnosis. Chen (1952), however, noted that the antigen responsible for haemagglutination, though present in old broth culture of *Y. pestis* as well as in the extracts of killed and dried plague bacilli, was absent in the protein fraction obtained by precipitation with sodium or ammonium sulphate, the nature of the substance being a polysaccharide.

The value of anti-protein sera: The various antiprotein sera provided

Table 34. Results of cross-agglutination tests of a few antiplague, anti-pseudotuberculosis and anti-aviseptica sera against *Y. pestis* and different yersnia strains

Antisera	*Y. pestis*	*Y. pseudotuberculosis*					*Y. aviseptic.*
	139/L	PR/I	PR/II	PR/III	PR/IV	No. 3245 '0'	
327/L virulent *Y. pestis*	400	800*	800*	100*	800*	400*	0
PR/I	0	1600	1600	200	800	100*	0
PR/II	0	1600	1600	400	200	400*	0
PR/III	0	200	100	800	200	—	0
PR/IV	0	200	200	200	800	—	0
Y. avisepticus	0	0	0	0	0	—	400

*Granular agglutination. The rest are of floccular type.

Table 35. Results of agglutination test of antiprotein sera against various plague and pseudotuberculosis organisms (minimum dilution 1 : 25)

Antiprotein sera	Virulent *Y. pestis* 139/L	Av. protective 53/Hav	Non-protective *Y. pestis*		*Y. pseudo-tuberculosis*	
			120/5 Hav	TRU	PR/I	PR/IV
L auto $P\frac{1}{3}$	400	200	0	0	0	0
L_5 $P\frac{1}{3}$	200	25	0	0	±	±
L_5 P ($\frac{1}{2}$-$\frac{1}{3}$)	0	0	50	25	25	25
L_5 $P\frac{1}{2}$	100	100	25	25	25	25
53/Hav $P\frac{1}{3}$	100	100	25 (f)	0	0	0
53/Hav $P\frac{1}{2}$-$\frac{1}{3}$	0	0	100	25	25	25 (f)
120/5 Hav $P\frac{1}{3}$	0	0	400	100	25	25
120/5 Hav P ($\frac{1}{2}$-$\frac{1}{3}$)	0	0	100	±	25	50
TRU $P\frac{1}{2}$	0	0	0	50	25	50
PR/I $P\frac{1}{3}$	0	0	25	50	50	25
PR/I P ($\frac{1}{2}$-$\frac{1}{3}$)	0	0	25	50	50	50
PR/IV $P\frac{1}{3}$	0	0	25	±	25	50
PR/IV P ($\frac{1}{2}$-$\frac{1}{3}$)	0	0	50	±	50	50

were tested for agglutination against several organisms representing virulent, avirulent protective and non-protective plague and pseudotuberculosis strains (Table 35).

Identification of protective antigen of plague bacillus: Rabbits were immunised with vaccine prepared from various types of plague and pseudotuberculosis organism and the antisera recovered from them were used directly after absorption with certain antigen (such as TRU and PRI) for testing their protective power in mice. The test was done according to Sokhey (1936) and the results assessed by Read and Muench's method. For each test 5 doses of antisera and 5 mice for each dose were used.

The results of the test showed that the three antiplague sera *viz.*, (1) 337/L, (2) 53/Hav and (3) 337/L (heat killed) had higher potency than others. But the highest potency *i.e.*, requiring minimum dose for protection was given by the antiserum raised against virulent plague bacillus. The antisera provided by the heat-killed and phenolised antigens and by alcohol-killed antigen were definitely inferior. The other group of antisera gave no protection to mice against virulent plague infection even with a dose as high as 0.2 ml. Thus antigen A contained in the virulent plague and avirulent protective organism is the protective antigen and antigen B ($P\frac{1}{2}$-$\frac{1}{3}$) is the common antigen between *Y. pestis* and *Y. pseudotuberculosis* and non-protective plague strains.

The same two types of agglutination were discernible in this test *viz.*, with 139/L, 253/Hav it was frankly floccular, with TRU and PR/I and PR/IV it was granular while with 120/5 Hav (non-protective avirulent plague) it was mainly granular. The results show that L/auto $P\frac{1}{3}$ (33% Na_2SO_4 pre-

cipitate of water soluble extract of virulent plague strain) agglutinated the virulent and avirulent protective plague strains but not the avirulent non-protective and pseudotuberculosis strains. A similar result had also been obtained with L_5 $P\frac{1}{3}$ and 53/Hav $P\frac{1}{3}$ antisera against proteins from virulent and avirulent protective plague organisms respectively. On the other hand, the antisera against P ($\frac{1}{2}$-$\frac{1}{3}$) fraction of virulent plague strains and against all fractions of the aviruent non-protective and pseudotuberculosis strains did not agglutinate the virulent and the protective avirulent strains but agglutinated the non-protective avirulent plague and pseudotuberculosis strain. The low titre of the sera might be indicative of low immunising power of these protein fractions as compared with the protein fractions of the virulent and avirulent protective strains.

Precipitation

The precipitation test could not be made popular for want of proper antigen. Previous workers who utilized precipitation test for the study of plague and allied organisms could not differentiate between the plague and pseudotuberculosis organisms. The reason for this cross-reaction has already been elaborated *i.e.*, the presence of two antigens in the filtrate, one specific for plague organism and the other for pseudotuberculosis strains. The isolation of specific protein fraction by various workers described earlier opened the prospect of utilising the technique as well for (i) serodiagnosis of plague infection in human beings and rats; (ii) identification of plague strains; and (iii) quantitative estimation of potency of antiplague sera. These methods have been actually utilised for plague studies by the author (Seal, 1951c; 1954) by fractionating the specific soluble protein, namely $P\frac{1}{3}$ fraction (antigen A) was found to be highly specific being precipitated by the plague serum against either virulent or partially virulent plague strains and against its own antiserum to a titre as high as 1 in million dilution. The P ($\frac{1}{2}$-$\frac{1}{3}$) (antigen B) fraction was not found as highly immunogenic as $P\frac{1}{3}$ fraction. However, the direct and cross-precipitation tests with these fractions with various absorbed and unabsorbed antisera showed a close similarity between the agglutination and precipitation reactions and the plague organism as preserved in the laboratory could be classified into the same three types as by the agglutination tests. The precipitation tests also confirm that $P\frac{1}{3}$ fraction of the protective plague strains corresponds to the surface or envelope antigen (antigen A) and the P ($\frac{1}{2}$-$\frac{1}{3}$) fraction to the somatic antigen (antigen B). The precipitation reaction could thus be utilized for the same purpose as served by the agglutination test with an added advantage that the dried antigen could be usefully used for serodiagnosis in the absence of plague strains in the field investigation as will be shown later (Chapter XIII).

Also as noted earlier the water extracted protein fraction L/auto $P\frac{1}{3}$ can more easily pick up the virulent and avirulent protective plague strains and thus the controversy between the high and low titre is eliminated. Further-

more, the precipitation test (flocculation test) could be used with advantage for testing the potency of antiplague horse or rabbit sera and in estimating the immunity status against plague in the rodents by means of gel precipitation test (Seal, 1972 b).

Procedures for Precipitation Reactions

For direct and cross-precipitation test the specific protein, antigen A, diluted to contain 0.1 mg/ml (1 : 10,000) is distributed in Felix tubes in 0.5 ml amounts. Patients' sera diluted 1 : 10 upward are added in 0.5 ml amounts. The tubes are lightly shaken and incubated at 37°C for 2 h. A preliminary reading is taken and the tubes are placed in the refrigerator for the final reading next morning. The highest dilution showing visible precipitates is taken to be the titre of the serum.

Ring Precipitation Test

For quicker diagnosis, ring precipitation test may also be done in the following manner: Antigen A 1/1000 dilution is taken in Dreyer's tube and the patients' undiluted serum or 1 : 2 to 1 : 10 dilution are added on the top of the antigen by means of a capillary pipette. A precipitating ring develops in ½ h at room temperature. The filtrate of casein hydrolysate broth inoculated with the unknown organism or the water extractable filtrate of distilled water suspension of the unknown organism may also be used for both ring and tube precipitation test.

The precipitin titres of various antisera as obtained against L_2 $P\frac{1}{3}$ fraction (antigen A) are given in Table 36.

The homologous and cross-precipitation reactions of protein fractions against various antisera are given in Table 37.

Ring precipitation test was done with absorbed sera against L_2 $P\frac{1}{3}$ (1 : 1000).

The ring precipitation reactions between the various protein fractions of the organism and anti-protein sera were compared. The results of direct and cross-precipitation tests between different protein fractions showed that $P\frac{1}{3}$ fraction of virulent and avirulent protective *Y. pestis* and 50/Hav behaved in the same manner in respect of agglutination as the surface antigen of the same organism, having shown cross-precipitation with each other's homologous and $P\frac{1}{2}$ protein antisera but not with the antisera against non-protective avirulent plague strains TRU and 53/Hav and pseudotuberculosis strains or their respective protein fractions. A low grade reaction of 120/5Hav antiserum against L_5 $P\frac{1}{3}$ is perhaps due to the existance of some residual surface antigen and in this respect also it behaved in the same manner on the agglutination reactions. The $P\frac{1}{3}$ fraction of the avirulent non-protective strains TRU and 120/5 Hav and of the pseudotuberculosis strains, on the other hand, gave cross precipitation reaction with antisera against the organism and their protein fractions but not with the antisera against virulent and avirulent protective strains against their $P\frac{1}{3}$ fractions.

Table 36. Precipitin titres of various antisera against L_2 $P\frac{1}{3}$ fraction

Antisera	Live antigen used for immunisation	Titre of L_2 $P\frac{1}{3}$
54/H (rabbit)	Virulent *Y. pestis*	2,000,000
H/23 of 2.11.41	Virulent *Y. pestis*, vaccine filtrate and relatively avirulent E.V.	1,000,000
H 27 of ,,	Virulent *Y. pestis* vaccine filtrate and relatively avirulent TjS	1,000,000
H 45 of ,,	Relatively avirulent TjS only	100,000
H 44 of ,,	-do-	50,000
Rabbit EV	Relatively avirulent *Y. pestis* E.V.	500,000
Rabit TjS	Avirulent protective *Y. pestis*	500,000
Rabbit TjS	Avirulent *Y. pestis* rough	200,000
Rabbit TRU	Avirulent non-protective *Y. pestis*	Cloudy only
Rabbit (PR/I)	Pseudotuberculosis strain	-do-
Rabbit (PR/II)	-do-	-do-

The results described here thus correspond exactly with those obtained in respect of agglutination. Thus the plague organisms can be divided into the same three groups by means of precipitation reaction carried out with specific protein fractions *viz.*, (a) Those whose antisera precipitate $P\frac{1}{3}$ fraction of virulent *Y. pestis* vaccine filtrate to a high titre; (b) those whose antisera precipitate this protein fraction only to a low titre; and (c) those whose antisera do not precipitate the protein fraction at all. The only difference between virulent and avirulent and protective plague strains is that $P\frac{1}{3}$ fraction of 53/Hav (av. protective) cross reacted with PR/I and PR/IV antisera at a very low titre (20,000), while a similar fraction from virulent plague strains showed negative reaction with those sera.

Antigenic Structure and the Protective Strains of Y. pestis

From the results discussed above it became evident that there are mainly two antigens in the plague bacillus (antigen A, $P\frac{1}{3}$ fraction; and antigen B, $P\frac{1}{2}$ fraction) and that both the antigens are present in 50 per cent Na_2SO_4 precipitate of the supernatant of virulent plague strains *e.g.*, L_5 $P\frac{1}{2}$.

Since the $P\frac{1}{3}$ protein has been found to be the mouse-protective fraction as noted earlier (Seal, 1953) it cantains protective antigen against plague bacillus and corresponds to its surface antigen. A similar observation had been made by Bhatnagar (1940) and Shrivastava (1940). In their experimental study of the cellular immunity to *Y. pestis* infection, the surface antigen was found responsible for the specific plasmatocytic response as well as protection, whereas the somatic antigen did neither give this specific cellular response nor afford protection to the animals against plague infection.

Table 37. Homologous and cross precipitation reactions of protein fractions against various antisera (titre in thousands)

Protein	L/auto $P\frac{1}{3}$	L_5 $P\frac{1}{3}$	L_5 $P(\frac{1}{2}\text{-}\frac{1}{3})$	337/L (virulent)	53/Hav av. protective	120/5/Hav (av. non-protective)	PR (I) Pseudotuberculosis	PR (IV) Pseudotuberculosis
L/auto $P\frac{1}{3}$	500	500	0	1000	1000	0	0	0
L/auto $P\frac{1}{2}\text{-}\frac{1}{3}$	20	20	0	50	50	0	0	0
L_5 $P\frac{1}{3}$	250	250	0	1000	500	50	±	±
L_5 $P(\frac{1}{2}\text{-}\frac{1}{3})$	±	20	50	100	50	20	50	50
L_5 $P\frac{1}{2}$	100	100	20	500	250	50	20	20
53/Hav $P\frac{1}{3}$	100	100	±	1000	1000	50	50	50
120/5 Hav $P\frac{1}{3}$	0	0	±	100	50	250	100	50
PR (I) $\frac{1}{2}$	0	0	C	C	C	20	50	50
PR (IV) $\frac{1}{2}$	0	0	C	C	C	20	50	50

0 = /20,000; C = cloudy; ± = doubtful.

Precipitation Method for Rapid Diagnosis of Rodent Plague

Cambosu (1928), Tumanosky (1939), Menzes (1940) and Larsen *et al* (1951) used precipitation test for diagnosis of rodent plague. The antigen is prepared from the tissue suspensions of animals which died of suspected plague, and sterilised by diethyl ether and is tested against a high titre anti-plague serum. Precipatation test with the antigens prepared from such tissues kept at 37°C as long as 14 wk also showed positive results. The test by Seal (1953) yielded better results with $P\frac{1}{3}$ specific antiserum so far as the plague infection is concerned.

Complement Fixation

Among the earlier workers, who had used complement-fixation test for diagnosis of plague were Strong (1908), Moses (1909) and Damperoff (1910). They used either bacillary suspension or extracts as antigen. But the antibodies could be found only after fifth day, though some workers (Shchastny, 1912) could not report this earlier than the second week. Its usefulness for diagnosis of human plague was noted by Joltrain (1936), particularly in case of convalescents or patients with mild symptoms, as the sero-agglutination test was inconclusive due to auto-agglutination. Simar (1921) and Dickie (1926) also tried this method and but the results were not always satisfactory. Chen *et al* (1952) successfully used complement-fixation test with plague fraction I antigen for detection plague infection in human convalescents, immunised animals and in the tissues of animals supposed to have died of plague infection. The test has also been used to differentiate between plague and pseudotuberculosis strains (Mitin, 1937; Haas, 1933) but the results were divergent. Greval and Dalal (1933) obtained specific graded results using filtered supernatant of Haffkine Plague Vaccine against antiplague sera raised in sheep, buffalo and bullock but Wats and Wagle (1935) did not find this technique sensitive enough. The latter used phage-lysed growth of *Y. pestis* in papain-digest broth with no better result.

The author (Seal, 1953) utilised specific soluble plague protein for complement fixation test. No cross-reaction was noted with anti-sera against either the protein fractions of the whole of avirulent non-protective plague or pseudotuberculosis strains. Similar study was also made by Chen, Quan and Meyer (1952) with a highly purified soluble protein antigen of *P. pestis* isolated by Baker *et al* (1947). According to them the method of complement fixation test, besides being of value for retrospective diagnosis of human plague, it is apt to be of importance for the diagnosis of rodent plague in the field especially when the isolation at autopsy is rendered difficult by contamination or decomposition. However, following the discovery of gel-precipitation test by Seal (1972 a), a much simpler method, the complicated complement-fixation test, for diagnosis of wild rodent plague remained of no use.

Technique of Complement Fixation Test

Antigen A ($P\frac{1}{3}$ fraction) is diluted to 1 : 4000 or 1 : 5000 in 1 per cent NaCl solution. The test sera are inactivated by heating in a water bath at 54°C for 10 min. Fresh complement is obtained by bleeding guineapigs each day and is used after titration as in the usual case of Wasserman test for syphilis.

Antibody titration: Tubes are placed in a series and 0.25 ml of various dilutions (1 : 4 to 1 : 200) of inactivated antisera is added to them, followed by 0.25 ml of 1 : 400 to 1 : 5000 dilution of antigen solution and 0.25 ml of $1\frac{1}{2}$ to 2 MHD of complement. The contents are then mixed well by shaking and the tubes incubated at 0 $\pm$ 3°C for $\frac{1}{2}$ h, put at room temperature for half an hour and then at 37°C for half an hour. The sensitized cells, prepared by the method of Greval (1946) were then added in 0.25 ml amounts, the tubes shaken and incubated at 37°C for half an hour. After a preliminary reading the tubes are left in the refrigerator overnight for the final reading to be taken next morning. Each series of tests is to be accompanied by serum, antigen, serum and antigen and sensitized cells control. Complement fixation is indicated by ++++ or '4' and complete haemolysis by 'O' and partial fixation by +++ or '3', ++ or '2' and + or '1' according to the states of hemolysed cells settle at the bottom.

The antiplague horse sera H 23 was found anti-complementary at and below 1 : 50 dilution. The test was therefore set up with 1 : 100, 1 : 200 and 1 : 300 of the antiserum against 1 : 5000 diltuion of the various protein solutions. The results are given in Table 38.

The result with antiplague horse serum was not entirely satisfactory although the reactions were obviously specific and the serum gave ring test positive against all the three proteins. The corresponding antiprotein rabbit sera, on the other hand, reacted specifically with the protein isolated from the protective plague strains, the antiserum Al reacting more strongly *i.e.*, with higher dilution of antigen than the other two and cross-reaction with the proteins from non-protective plague as well as pseudotuberculosis strains were completely avoided, but the test failed to distinguish virulent from avirulent protective strains.

Since it was of interest to find out how the antisera against their respective whole organism behave against these protein fractions the materials were used for complement fixation test of which the results are given in Table 39. The results indicate that the response of antiserum produced against the whole virulent plague organism was slightly broader than that of the antiserum against the $P\frac{1}{3}$ protein fraction from the filtrate of the vaccine prepared with the same organism. For instance, antiserum against virulent plague bacillus 139/L cross reacted at low dilution with the protein fraction of avirulent non-protective strain 120/5H and pseudotuberculosis strain PR (I), probably due to the presence of antigen B. This observation finds support in the behaviour of both Al and A2 anti-protein sera (containing only the antibody against antigen A) giving a negative reaction with the protein

Table 38. Complement fixation tests of H 23 antiplague horse serum and L auto $P\frac{1}{3}$, $P\frac{1}{2}$ and 53/Hav $P\frac{1}{3}$ antiprotein rabbit sera with various protein fractions

Antigen	Antigen 1 : 1000	Antigen 1 : 4000		Antigen 1 : 1000				Antigen 1 : 5000	
	H 23 antiplague horse serum*	Al (L_5 $P\frac{1}{2}$) antiprotein rabbit serum**		A2 (L auto $P\frac{1}{2}$) antiprotein rabbit serum				A3 (53/Hav $P\frac{1}{3}$) antiprotein rabbit serum	
	1 : 100	1 : 10	1 : 25	1 : 10	1 : 25	1 : 50	1 : 100	1 : 10	1 : 25
L_5 $P\frac{1}{3}$	1	3	2	4	3	2	1	2	±
L/auto $P\frac{1}{3}$	±	2	±	4	3	2	1	2	±
53/Hav $P\frac{1}{3}$	±	2	1	4	3	2	1	3	2
TRU $P\frac{1}{2}$	0	0	0	0	0	0	0	0	0
PR/I $P\frac{1}{2}$	0	0	0	0	0	0	0	0	0
PR/IV $P\frac{1}{2}$	0	0	0	0	0	0	0	0	0

1 = +; 2 = ++; 3 = +++; 4 = ++++; 0 = negative; ± = doubtful; A1 = Protein isolates from virulent plague strains; A2 = From autolysates of *Y. pestis*; and A3 = From avirulent protective plague strains.

*The results were negative at 1 : 200 and 1 : 300 dilutions.

**The results were negative with all antigens at 1 : 100 and in all except with L_5 $P\frac{1}{3}$ at 1 : 50.

Results were negative at 1 : 50 and 1 : 100 dilutions.

Antigen controls gave negative results throughout.

Table 39. Complement-fixation tests of 139/L (virulent), 53/Hav (av. protective) and TRU (av. non-protective) antiplague and PR (I) and PR (IV) anti-pseudotuberculosis rabbit sera with various protein fractions (1 : 5000)

Antigen description	No.	139/L antiplague rabbit sera				53/Hav antiplague rabbit sera				TRU antiplague (non-protective) rabbit sera				PR (I) anti-pseudotuberculosis rabbit sera	
		1 : 25	1 : 50	1 : 100	1 : 200	1 : 25	1 : 50	1 : 100	1 : 200	1 : 25	1 : 50	1 : 100	1 : 200	1 : 25	1 : 50
L_s $P_{\frac{1}{3}}$	A1	4	4	3	1	+±	1	0	0	±	0	0	2/3	2	1
L/auto $P_{\frac{1}{3}}$	A2	4	3	±	0	+±	1	0	0	±	0	0	0	0	0
53/Hav $P_{\frac{1}{3}}$	A3	4	4	4	3	4	3	+±	±	±	0	0	0	0	0
120/5 Hav $P_{\frac{1}{3}}$	B1	2	1	0	0	1	0	0	0	1	0	0	0	0	0
TRU $P_{\frac{1}{2}}$	B2	±	0	0	0	±	0	0	0	2	1	±	0	1	±
PR (I) $P_{\frac{1}{2}}$	B3	1	0	0	0	1	0	0	0	±	0	0	0	4	3
PR (IV) $P_{\frac{1}{2}}$	B4	0	0	0	0	0	0	0	0	0	0	0	0	0	0

0 = negative; 1 = +; 2 = + +; 3 = + + +; 4 = + + + +; ± = doubtful; 1.5 = + ±.

PR (IV) $P_{\frac{1}{2}}$ antigen gave negative results in all except with 1 : 25 (reading = 2) and 1 : 50 (reading = 1) PR (IV) anti-pseudotuberculosis rabbit sera. 1 : 100 of PR (I) as well as PR (IV) anti-pseudotuberculosis rabbit sera gave negative results throughout. Similarly, results were negative with 1 : 200 of PR (IV) anti-pseudotuberculosis rabbit sera (and also with antigen control).

fractions B_1, B_2 and B_3 and B_4.

Anti-serum against avirulent protective strain 53/Hav behaved like 139/L antiserum. TRU antiserum reacted with the protein fractions of another avirulent non-protective plague strain 120/5Hav at a low dilution but not with virulent or avirulent protective plague strains. Also protein fraction B_2 or TRU $P\frac{1}{2}$ cross reacted with PR (I) antiserum. Thus complement fixation test also failed to distinguish between virulent from avirulent protective plague strains.

For testing antibody in human sera higher antigen dilution to the extent of 1 in 10,000 to 1 in 20,000 is sometimes necessary. The sera of 20 convalescent patients of confirmed plague cases tested by this technique in 1949 gave 83 per cent positive results.

Immunogenic Properties of Antigens and Vaccines

The immunogenic properties of several vaccines and protein fractions and the mouse protective powers of the antiprotein sera were evaluated through the mouse protection test of Sokhey (1939b) standardised on the basis of Reed and Muench's (1938) statistical evaluation of the dose at 50 per cent level of deaths. The immunising power of each protein fraction was compared with that of its parent vaccines.

Test Dose of Vaccines, Proteins and Antisera

The protective dose of Haffkine plague vaccine usually varied between 0.004 and 0.006 ml (Sokhey, 1939b). The yield of each protein fraction per minimum protective dose of the corresponding vaccine was calculated from the total yield of the fraction in the entire lot of that vaccine. Approximately three to five doses were used in the same manner as the vaccines. In a few instances more closely set doses were used than those of the trials, in order to obtain an endpoint. For assessing the protective values of antisera, several doses were used in geometrical progression close to the probable protective dose determined by a preliminary test.

Test Materials

A sample of 100 to 200 ml was kept apart from each of the following lots of live or killed vaccines and was preserved in the cold until it was used to determine the minimum mouse-protective dose: (1) killed vaccine prepared from the virulent plague strain 327/L grown at 37°C for 72 h, (2) to (4): Heat killed vaccine prepared from each of the virulent plague strains 139/L, 337/L and from the avirulent plague strain 53/Hav, grown at 28°C for 3 wk, (5) to (7): Live vaccines prepared from the avirulent non-protective plague strains-120/5Hav and TRU grown at 37°C for 72 h, (8) Killed vaccines prepared from *Y. pseudotuberculosis* strains-PR (I), grown at 28°C for 3 wk. Enriched casein hydrolysate broth was the culture medium for all except (1) for which casein hydrolysate agar developed by the author (Seal, 1943; Seal and Mukherjee, 1950).

The protein fractions tested were L_5 $P\frac{1}{3}$, L/auto $P\frac{1}{3}$, L_5 $P\frac{1}{2}$, L_3 $P\frac{1}{3}$ and L_3 $P\frac{1}{2}$ numbered respectively as A_1, A_2, A_4, A_5 and A_6. The yield of the protein fraction $P\frac{1}{2}$-$\frac{1}{3}$ (A_7) was always very poor and the quantity was insufficient for all the tests and hence these were carried out with the other fractions except with A_7 or L/auto $P\frac{1}{2}$-$\frac{1}{3}$ or L_5 $P\frac{1}{2}$-$\frac{1}{3}$. The five antisera tested were : A_1, A_2, A_4, A_5 and A_6, the total doses used for immunising the rabbits being 40, 38, 36, 39 and 37 mg respectively. The test dose of each antisera was 1 ml against a test infective dose of 118-127 organisms (mouse m.l.d. being 6-12 *Y. pestis*). The results of mouse-protective test are given in Table 40.

Immunization Values of Various Plague Vaccines

The minimum mouse-protective doses of the first four vaccines were: 0.0056, 0.0053, 0.0056 and 0.006 ml respectively (Sokhey, 1939b) and that of live 53/Hav vaccine was estimated to be 875-1000 organism. The other two live vaccines—120/5Hav and TRU did not confer any protection with 10 and 22 million organisms respectively; while the PR (I) killed vaccine, even when it contained 0 + million organisms, failed to protect against *Y. pestis*.

Immunogenic Values of Protein Fractions

The mouse-protective values of the fractions tested are given in Table 40 Protein fraction A_1 protected mice in doses much smaller (actually half) than the actual yield per minimum protective dose of the corresponding whole vaccine; while A_4 gave protection at a dose slightly less (0.8 parts) than the equivalent yield. The doses of A_5 and A_6 fractions were five times the equivalent yield from the vaccine. The minimum mouse protective dose of L/auto A_2 was two and half times the equivalent yield of the mother vaccine. All the mice given A7 and 2 of 5 given B5 survived. Serologically the former was similar to the protective fraction A2, while the latter was related to the protein fractions isolated from the non-protective plague and pseudotuberculosis strains.

Mouse Protective Values of Certain Anti-protein Sera

The numbers of mice surviving out of 5 mice used in each test of A1, A2, A5 were 3, 4 and 1 respectively and none among the 10 control mice. In other words A1 or L_5 $P\frac{1}{5}$ antiserum like A_1 protein gave comparatively better protection than the other two antisera, of which A5 or L_3 $P\frac{1}{3}$ antisera proved practically non-protective in the doses used and A2 or L/auto $P\frac{1}{3}$ antiserum intermediate between the other two. This result supports the main conclusion arrived at in connection with the immunogenicity of these proteins.

The above results have been obtained with minimum dose. It may fairly be concluded that with increased dose or repeat dose as is done in case of Haffkine plague vaccine, the protective power would be greatly enhanced with L_5 $P\frac{1}{3}$ or antigen A protein fraction. Thus the protective power of the Haffkine plague vaccine lying in the protein fractions of its supernatant $P\frac{1}{2}$ fraction (antigen A) of the first or the second group of organism (av. protec-

Table 40. Mouse protection doses of plague protein fractions compared to those of the Haffkine plague vaccine

Protein fractions	No.	Yield per ml of vaccine, mg	Mouse-protective dose of Haffkine plague vaccine, mg	Yield of protein for mouse-protective dose of vaccine, mg	Doses of protein fractions tried, mg					Test dose of virulent *P. pestis*
Descriptive					1	2	3	4	5	
L_5 $P\frac{1}{3}$	A1	0.24	0.0053	0.0012	0.00024	0.00048	0.00096	—	—	127
L/auto $P\frac{1}{3}$	A2	0.17	0.0056*	0.00095	0.001	0.005	0.025	—	—	127
L_5 $P\frac{1}{2}$	A4	0.287	0.0053	0.0015	0.0003	0.006	0.0012	0.0024	0.0048	127
L_3 $P\frac{1}{3}$	A5	0.176	0.0056	0.001	0.0008	0.0016	0.0032	0.005	—	118
L_3 $P\frac{1}{2}$	A6	0.246	0.0056	0.00138	0.001064	0.002128	0.004256	0.00665	—	118

*This CH (casein hydrolysate) agar vaccine, the rest were CH broth vaccine.

Result according to dose					Control	Average mouse protective dose, mg	Protective dose of multiples or fractions of equivalent yield
1	2	3	4	5			
2/5	3/5	1/5	—	—	5/5	0.00055	0.5
4/5	1/5	0/5	—	—	5/5	0.0025	2.5
3/5	2/5	3/5	4/5	0/5	5/5	0.0012	0.8
5/5	5/5	4/5	3/5	—	5/5	0.005	5.0
5/5	5/5	4/5	3/5	—	5/5	0.00665 (approx.)	5.0

tive) is fully responsible for the mouse protection given by the mother vaccine. Its immunogenic value also depends on the quality of the medium used. The $P\frac{1}{2}$-$\frac{1}{3}$ fraction (antigen B) on the other hand, gives practically no protection even with heavy doses. The results are also supported by the mouse protection given by the corresponding antisera (anti-protein).

These studies also led to the conclusion that $P\frac{1}{2}$ fraction of the virulent plague organism or the vaccine prepared from it would be the choice of immunising agent instead of the liquid vaccine, the potency of which deteriorates with time while the protein fraction has been utilised for immunological work after keeping it for 25 yr in the refrigerator without loss of potency.

Qualitative and Quantitative Agglutination and Precipitation Tests

For quantitative measurement of the potency of antiplague vaccines and sera the biological test as standardised by Sokhey and others had only been in use. But this method is time-consuming (about a month) and requires maintenance of a standard herd of white mice and a well equipped laboratory and technical staff to detect the cause of death of test animals with consequent high cost and skill. A shorter and quicker method of agglutination or precipitation test could not be undertaken owing to the inherent difficulties in carrying out these tests for want of a stable suspension in case of agglutination and suitable antigen in case of precipitation. Establishment of an *in vitro* method having a quantitative relationship with mouse protective potency would therefore be of great value in shortening the period and reducing the cost of such tests.

The success of evolving a suitable culture medium and isolation of specific soluble protein from the plague organism and allied organisms finally facilitated the development and utilisation of quantitative agglutinin and precipitin nitrogen method for standardising assay of potency of antiplague sera and vaccine within a much shorter time than the biological test and with good accuracy. The analytical method developed by Heidelberger and his co-workers (1929, 1933, 1934, 1935a and 1935b) during their studies on antigen antibody system in order to assess the potency of anti-pneumococcic sera, was utilised for assessment of potency of antiplague sera by the estimation of agglutinin or precipitin nitrogen. The suspension of virulent plague organism being the antigen used for agglutinin N and specific soluble protein like L_5 $P\frac{1}{3}$ free from any nonspecific N being obtained from a protein free medium for precipitin N estimation. Micro-Kjeldahl method as modified by Heidelberger and Kabat (1934, 1936-38) were used for estimating the nitrogen.

In the present study quantitative agglutinin nitrogen values for various absorbed and unabsorbed antiplague horse and rabbit sera were determined and the results compared with their corresponding mouse protection doses.

The test values were determined using the following formula:

$$\text{Agglutinin N per ml} = \frac{\text{Total N (mg)} - \text{Antigen N (mg)}}{\text{Amount of serum (ml)}} \times \text{times the serum is diluted}$$

$$\text{Precipitin N values per ml of antiserum (in mg)} = \frac{\text{Total N (mg)} - \text{Antigen N (mg)}}{\text{Amount of serum (ml)}} \times \text{times the serum is diluted}$$

The test was fully standardised on the basis of the effects of temperature of incubation and of the use of constant serum against variable antigen. As with other horse serum system the highest quantity of antibody N was obtained when all the manipulations were carried out at $0° \pm 4°C$ and not at room temperature or at 37°C for 2 h followed by overnight refrigeration. With rabbit antisera, however, the yield was not significantly effected by a preliminary incubation for 2 h at 37°C or at room temperature, but the results at 0°C for 48 h were more consistent. All tests were carried out in duplicate at $0° \pm 4°C$ and sera were incubated in the refrigerator for 48 h. The quality of antigen was also an important factor.

The antigen used was fully standardised and repeated testing of the antiserum H 23 of Nov. 28, 1941 with this antigen yielded the same antibody N value each time. Based on the findings of these tests, similar tests were carried out with unabsorbed antisera with their corresponding mouse protective doses arranged in certain serial order and the results are given in Table 41.

Quantitative Precipitin N Values of Various Horse and Rabbit Antiplague Sera

It is seen that the number of antibody N increases with the immunisation of horses up to a certain level and then it seems to deteriorate even with continued injection as illustrated in case of H 27. It is also apparent that the quantitative correlation with mouse-protective doses is better established through the estimation of quantitative precipitin N and agglutinin N values which vary inversely with the mouse-protective values the figures for agglutinin N values being consistently higher than those of precipitin N and decreasing and increasing in a regular order. Also, there is probably a peak stage in immunisation which should be reached before a full bleeding is allowed.

The highest antibody N value was given by the rabbit antiserum produced against the live virulent plague bacillus and the nearest approximation to this value was reached by a serum raised against a relatively avirulent plague strain. The sera against the heat-killed antigen were nearly as good but very heavy doses (many thousand times bigger than that of the virulent *Y. pestis* used for immunisation). Poor results were, however, obtained with alcohol-killed and heat-killed carbolised antigen.

Quantitative Agglutinin N Values of Various Horse and Rabbit Antiplague Sera

As expected the various antisera gave quantitative N values which are consistently higher than the corresponding quantitative precipitin N values.

Table 41. Agglutination and precipitation titres and quantitative agglutinin and precipitin N values of several antiplague horse and rabbit antisera in relation to their mouse-protective doses arranged in descending values

Sl no.	Antiplague sera	Antigen used for immunisation	Qualitative		Quantitative		
			Agg. titre against virulent *P. pestis*	Precipitin titre against L_5 P1/3	Mouse protective doses, ml	Precipitin N values, mg/ml	Agglutinin N values, mg/ml
1.	R. 83 (rabbit)	Alcohol-treated vir. *Y. pestis*	200	40	0.8	0.20	0.24
2.	R. 100 (rabbit)	Vir. *P. pestis* 337/L, heat killed and phenolised	400	80	0.62	0.44	0.78
3.	H. 27 (horse) of Nov. 2, 1941	Life avirulent and then vir. *Y. pestis* + vaccine filtrate	400	80 (160±)	0.40	0.37	0.80
4.	H. 27 (horse) of May 2, 1942	-do-	200*	—	0.068	1.38	1.68
5.	H. 24 (horse) of Nov. 28, 1941	-do- + Live av. TjS	800	640	0.18	0.92	1.28
6.	H. 27 (horse) of Nov. 5, 1942	Live av. and then vir. *Y. pestis* + vaccine filtrate	400	—	0.10	1.04	1.32
7.	H. 23 (horse) of Nov. 28, 1941	-do- E.V. strain	600	640 (1280±)	0.075	1.30	2.00
8.	R. 33A (rabbit)	Live av. protective *Y. pestis* 53/Hav	800	320 (640±)	0.037	1.68	1.96

(*Contd.*)

Table 41. *Continued*

9. R. 37 (rabbit)	Vir. *Y. pestis* live	800	320 (640±)	0.033	1.92	2.24
10. R. 104 (rabbit)	Vir. *Y. pestis* boiled for 30 min.	0	0 (10±)	No protection	0	0.60

Vir = virulent; av. = avirulent; TjS = Java plague strain (Tjiwielej smooth of others).

** E.V. = Madagascar av. protective plague strain of Girard and Robic.

* Aparently there was an error in reading as on repitition the titre was found to be 400+.

In order to evaluate the role of somatic antibody for this difference, the two antisera-337/L and 53H/av. were absorbed with an avirulent non-protective plague TRU strain and 53H/av antiserum with the pseudotuberculosis strain (PR/I) followed by the reestimation of agglutinin N value. The strain TRU absorbed the same quantity of antibody (0.76 mg) from both the antisera, while the agglutinin N values, lost due to absorption with the pseudotuberculosis strain PR (I), was only 0.56 mg. It may be remembered here that TRU is a rough dissocient of a plague strain whereas PR (I) is only a member of the above group *Y. pseudotuberculosis*. The quantitative agglutinin N values and the corresponding mouse-protective doses of the above antisera, before and after absorption are compared in Table 42.

The results of absorption given in Table 42 are consistent though the agglutinin N values are lower than the corresponding precipitin nitrogen values. Since after absorption with non-protective strains the mouse-protective doses were increased it may be that in protection against plague somatic antigen may also have some role (as an antitoxic agent) as suggested by Jawetz and Meyer (1943).

Statistical Analysis of the Data

Coefficient of Correlation and Regression Lines

The practical utility of quantitative precipitin N or quantitative agglutinin N will depend on the precision of its quantal correspondence with the mouse-protective dose. A large number of tests performed permitted the calculation on coefficients of correlation and fitting of regression lines. Both the agglutinin N and precipitin N values vary inversely with the mouse protective dose, while the figures for agglutinin N of the unabsorbed sera being consistently higher, the precipitin N, kept decreasing or increasing regularly.

The first and second degree regression equations which have been obtained for the mouse protective units from precipitin N values are (1) $X = 16.30\ Y - 5.61$, and (2) $X = 2.06 - 4.88\ Y - 10.58\ Y^2$ where X represents

Table 42. Quantitative agglutinin N values and mouse-protective doses of certain antiplague sera before and after absorption with TRU and PR (I) strains

Absorption antisera	Agglutinin N, mg			Mouse-protective doses, ml			Precipitin N values of unabsorbed sera, mg
	BA	AA	Difference	BA	AA	Difference	
1. 337/1 absorbed with TRU	2.24	1.48	0.76	0.033	0.068	0.035	1.92
2. 53/Hav absorbed with TRU	1.96	1.20	0.76	0.037	0.096	0.059	1.68
3. 53/Hav obsorbed with PR (I)	1.96	1.40	0.56	0.037	0.080	0.043	1.68

BA = before absorption; AA = after absorption.

the mouse-protective unit and the preciptin N values in mg/ml. Two such regression equations are also obtained for mouse protective units from agglutinin N values. These are (3) $X = 14.25\ Z - 7.46$ and (4) $-X = 0.946 - 1.962\ Z + 6.292\ Z^2$, where X represents the mouse protective units as above and Z the agglutinin N values in mg/ml. The linear equation which gives regression of precipitin N values on agglutinin N value is (5) $Y = 0.8247\ Z - 0.0776$, where Y represents the precipitin N values and Z the agglutinin N values in mg/ml. The goodness of fit of these deduced regression formulae has been confirmed by analysis of variance. From this analysis it has been found that although the first degree regression of mouse protective units or precipitin N values is highly significant (even at 0.1% point), a second degree fitting shows a significant improvement on the former. The point of equivalence in this case, therefore does not follow a fixed proportional value and thus the second degree regression gives decidedly better relation of mouse protective units with precipitin N values, while in the case of regression of mouse protective units on agglutinin N values, the first degree equation gives significant relation but the degree fitting does not improve this relation as significantly as in the first case. In case of precipitin N values or agglutinin N values the analysis of variance shows a highly significant relation, as already indicated by their correlation coefficient. In fact, in the first case the linear regression explains 90 per cent of the variations, while the parabolic regression explains 98 per cent (residual variance = 1.62) as against 80 to 86 per cent (residual variance = 14.62) respectively to the second case.

From the analysis of data it is evident that agglutinin N values may be used for the purpose of estimating mouse protective units. The best estimated mouse protection units will be obtained from the precipitin N values through the second degree regression equation *viz.*, $Y = 2.06 - 4.88\ Y + 10.58\ Y^2$. It is also interesting to note that in the case of regression of precipitin N values and agglutinin N values, 93 per cent of the variation can be explained by fitting a linear relation. The relationship between the agglutinin N, precipitin N and mouse protective values by using 14 different antisera raised in horse and rabbits have been shown in Figs 34-36.

Thus a quantitative precipitin N test developed after Heidelberger and Kendall's technique with the $P\frac{1}{3}$ protein fraction, isolated from the supernatant of the Haffkine plague vaccine produced in casein hydrolysate enriched broth and a quantitative agglutinin N test carried out according to the Heidelberger and Kabats' technique with a standardised suspension of virulent plague organism grown in the same broth at 28°C for 72 h and killed by 0.1 per cent formalin, have been established with statistical evaluation. These quantitative methods permit quick titration of sera of animals for guiding the procedures at different stages of immunisation. A tentative mouse protective unit has been suggested which may be used as the basis for working at a method for standardisation of antiplague sera. Antisera produced in rabbits are found superior to that of antiplague horse sera.

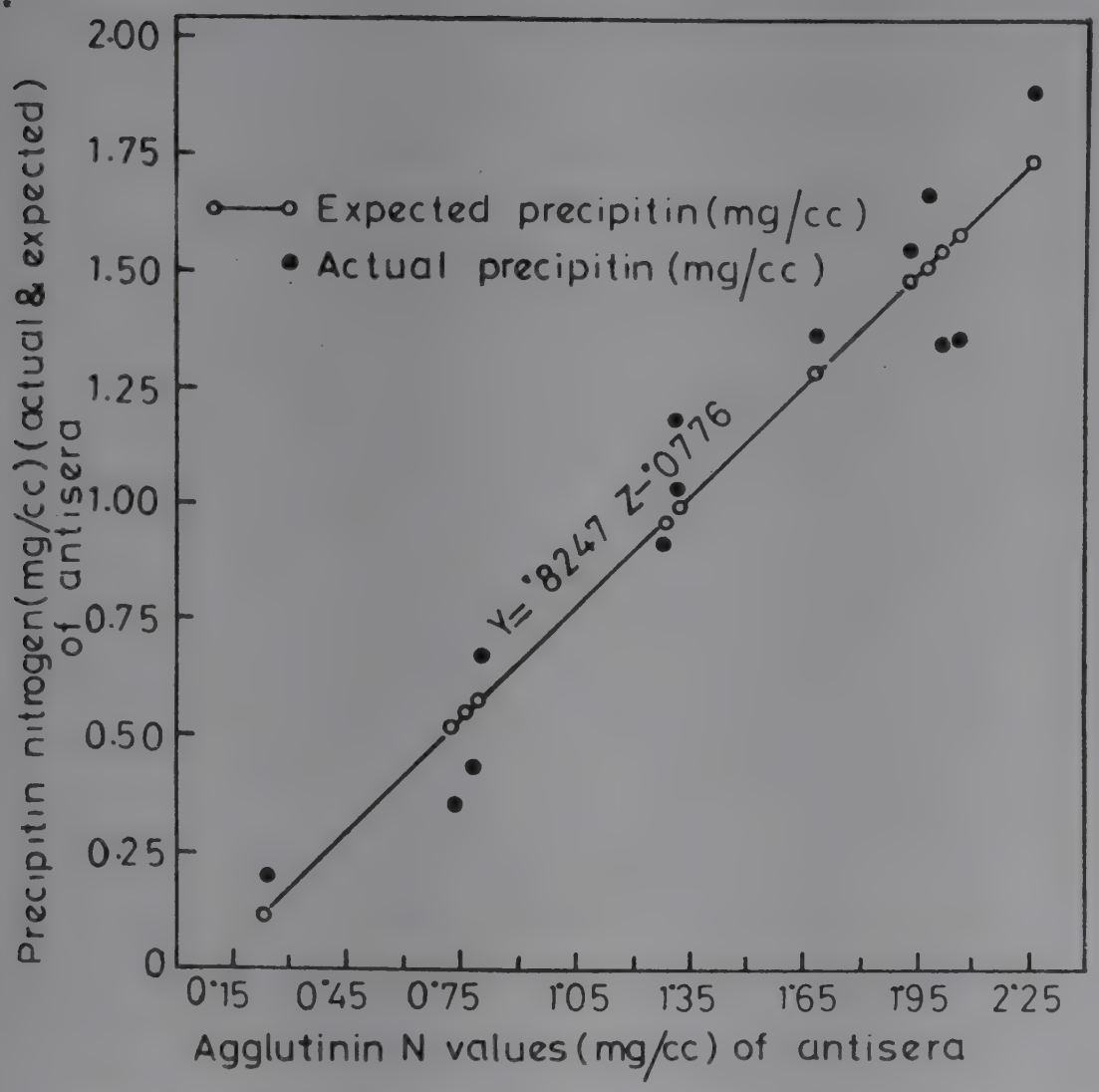

Fig. 34. Mouse-protection units of anti-plague sera in relation to their precipitin N value (mg/ml).

Flocculation Test (for titration of antiplague serum)

Advantage has also been taken of the specific plague proteins in developing a flocculation technique for estimating the potency of antiplague sera apart from quantitative agglutination and quantitative precipitation tests. Based on constant serum and variable antigen method between antigen A and the various horse and rabbit antiplague serum, the author successfully established the technique of flocculation appearing in 5 to 15 min at 45°C. A preliminary assessment of Lf values of the antiplague sera compared very favourably with the corresponding observed precipitin nitrogen values ($r = 0.9843$, highly significant). 1 Lf unit was proposed to be equivalent to 1 mg of specific protein, calculated from the optimum proportion figures of antigen and antiserum at which the first flocculation was observed. This flocculation method can now serve as the rough and ready *in vitro* test for the titration of antiplague serum. Perhaps this technique would also be useful in titrating the antitoxic potency of such sera.

Among other workers Girard (1941) tried to develop a diagnostic procedure by mixing 5 drops of patient's serum with 1 ml of plague endotoxin. Flocculation took place in positive case. Readings were taken after 30 min,

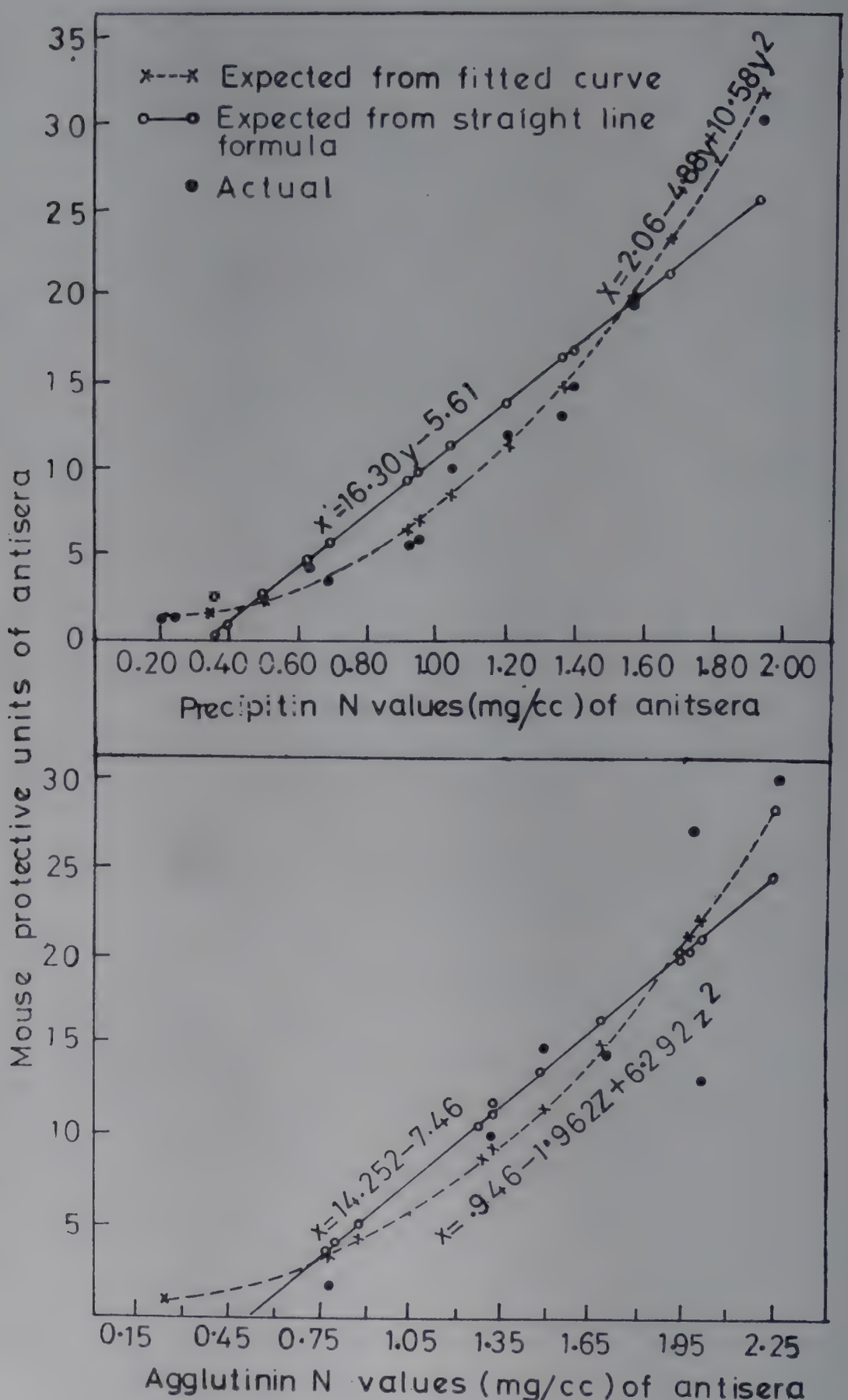
Expected from fitted curve
Expected from straight line formula
Actual
x=2·06−4·89y+10·58y²
x'=16·30y−5·61
Mouse protective units of antisera
Precipitin N values (mg/cc) of anitsera
0.20 0.40 0.60 0·80 1·00 1·20 1.40 1.60 1·80 2·00
x=14·252−7·46
x=·946−1·962z+6·292z²
Agglutinin N values (mg/cc) of antisera
0·15 0·45 0·75 1.05 1·35 1.65 1·95 2·25

Fig. 35. Mouse-protection units of anti-plague sera in relation to the agglutinin N values (mg/ml).

Fig. 36. Expected precipitin N values (mg/ml) of anti-plague sera in relation to the agglutinin N values (mg/ml).

3 and 24 h. The method developed for flocculation test used a constant antiserum (0.5) against variable antigen. In the absence of a standard antiserum it was tentatively decided to express the potency of the unknown serum corresponding to 1 mg of specific protein as 1 Lf units. Later when a standard antiserum is made available the Lf units may be assessed in terms of that standard. A protocol of the test is given in Table 43.

The Lf units according to the provisional standard at the outset have been calculated and compared with the quantitative nitrogen values in 7 antisera (Table 44).

Table 43. Antigen L_5 P1/3 (1 mg/ml) against 337/L antiplague serum raised against live *Y. pestis*

	Tube no.					Serum content ml	Antigen content ml
	1	2	3	4	5		
Antiserum R. 37 against 337/L *Y. pestis* 1/80 ml				0.5 (constant)			
Antigen L_5 P⅓ mg in 0.5 ml	0.0625	0.075	0.0875	0.1	0.1125	0.5 (saline)	0.1125
	cl	cl	F	(f)	cl	x	x

cl = cloudy; F = flocculation appearing earliest; (f) = flocculation after it appeared in the tube no. 3.

Table 44. Lf values of different antisera compared with the quantitative precipitin N values

Antisera	Amount of antigen, mg	Quantity of serum mg of antigen	Provisional Lf units	Calculated antibody N values/ml of serum, mg	Observed precipitin N values/ml of serum, mg
H23 of Haffkine Inst.	0.05	0.125	0.0	1.25	1.2
H27 of Haffkine Inst.	0.06	0.100	10.0	1.6	1.38
H29 of Haffkine Inst.	0.05	0.125	8.0	1.28	1.04
R33A (53/Hav)	0.08	0.083	12.0	1.92	1.68
R37 (337/L)	0.09	0.071	14.1	2.40	1.92
R99 (337/L) heat killed	0.06	0.100	10.1	1.60	1.56
R100 (307/L) heat killed and carbolised	0.02	0.330	3.0	0.48	0.44

The amount of serum was 0.00625 ml in each case.

CHAPTER X

Methods of Differentiation between *Yersinia pestis* and *Yersinia pseudotuberculosis*

In the epidemiology of plague it was of utmost importance to differentiate *Yersinia pseudotuberculosis* from *Yersinia pestis* both of which affect the rodent population which act as reservoir for both types of infection. Human infection with *Y. pseudotuberculosis* though rare has also been reported. Thus from the points of view of both rodent and human infection it was necessary to identify these organisms without any ambiguity or doubt. These two organisms resemble each other more than any other member of the yersinia family. The position was further complicated because there was much controversy about the plague organism itself. Although motility is a distinguishing feature of the pseudotuberculosis organism there are pseudotuberculosis strains which are non-motile due to loss of flagella. Controversy also existed in the results of sugar and glycerol fermentation tests. The author (Seal, 1952) made a critical examination of each type of test for differentiating the two organisms namely, culture medium, morphology (by staining of capsule and envelope), motility, biochemical and serological characteristics, agglutination, precipitation and absorption of agglutinins and precipitins, chemical antigenic structure and also pathogenicity and animal protection tests.

Classification of *Y. pseudotuberculosis* Organisms

Prevot (1948) proposed to classify pseudotuberculosis strains under the name 'Cilio-pasteurella' on the basis of its possessing flagella but non-flagellated forms have also been found as in case of *E. typhosus*. So the name *Pasteurella pseudotuberculosis* as proposed by Pfeiffer (1890) and Girard (1952) was retained. Later, however, the genus *Pasteurella* has been changed to *Yersinia*.

Culture Characteristics

The most suitable media for culture and maintenance of the organism as found by Seal (1952) are: freshly prepared 5 per cent rabbit blood agar

(Gore, 1929) or enriched casein hydrolysate broth prepared by Seal and Mukherji (1950) and casein hydrolysate agar (Seal 1950) in which the organism grows from a very small inoculum without any lag period. For differential test freshly isolated virulent *Y. pestis* shows no noticeable growth with a small inoculum on A-D agar at 37°C whereas *Y. pseudotuberculosis* shows fairly luxuriant growth. On the rabbit blood agar discrete colonies can be obtained even at the millionth dilution of a 48 hours culture of *Y. pestis* in nutrient broth at 37°C, while in 48 hours at 37°C no growth occurs in A-D agar in dilution higher at 1 in 100 (giving rare colonies at this dilution and a few at 1 : 10). But if the blood of a plague case is directly inoculated on nutrient or A-D agar, growth may occur due to the presence of blood on the medium.

This test is not valid for *Y. pestis* which has lost its virulence and protective antigen during maintenance in the laboratory. Such organisms have also been discovered by the author in live rats during the inter-epidemic or pre-epidemic period, which grow fairly well in nutrient agar and can easily be mistaken for *Y. pseudotuberculosis*.

Envelope Substance and Capsule

Virulent and avirulent protective *Y. pestis* develop envelope substance and capsule when grown on 5 per cent rabbit blood agar or enriched casein hydrolysate agar at 37°C for 48 h. Treated in a similar manner *Y. pseudotuberculosis* shows no capsule nor envelope substance (Figs. 37-39). The India Ink Method of Butt and Joy (1936) as modified by Bhatnagar (1940) for detection of envelope substance and the capsule staining method evolved by the author (Seal, 1959) give reliable results. Again, the tests are not valid for avirulent non-protective plague strains which like *Y. pseudotuberculosis* shows neither capsule nor the envelope substance.

Motility

Motility is a fundamental character of *Y. pseudotuberculosis* but it is absent in *Y. pestis*. The method used by Girard was by staining flagella, but in practice a stab culture in ½ per cent semi-solid agar, incubated between 22° and 25°C has been found convenient. A characteristic inverted pine-tree-like growth is given by *Y. pseudotuberculosis* as an evidence of motility, whereas non-motile plague strain remains as a single streak (Fig. 40). The seed culture used for this test is taken from 24 hours growth of the trial organism on 5 per cent rabbit blood agar at 37°C. The character of motility can, however, be suppressed by modifying the media or by changing the temperature of the incubation (Kessel and Overbrook, 1901; Arkwright, 1927).

Biochemical Reactions

Y. pseudotuberculosis gives positive H_2S test and reduces both malachite green and methylene blue (1 : 50,000) and turns litmus milk alkaline while *Y. pestis* shows no change in the litmus milk and does not reduce malachite

Figs. 37-38. Diagrammatic representation of three parts of virulent *Y. pestis* by different research workers.

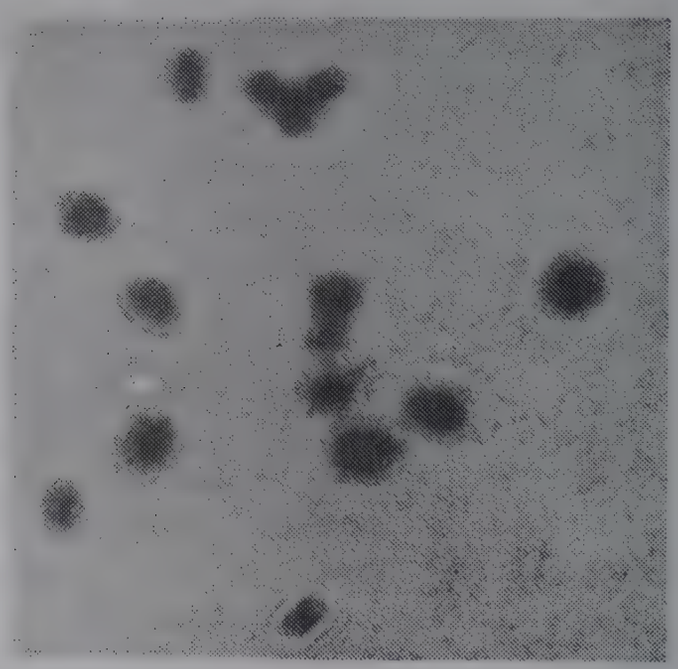

Fig. 39. Envelope and capsule of *Y. pestis* by differential stain.

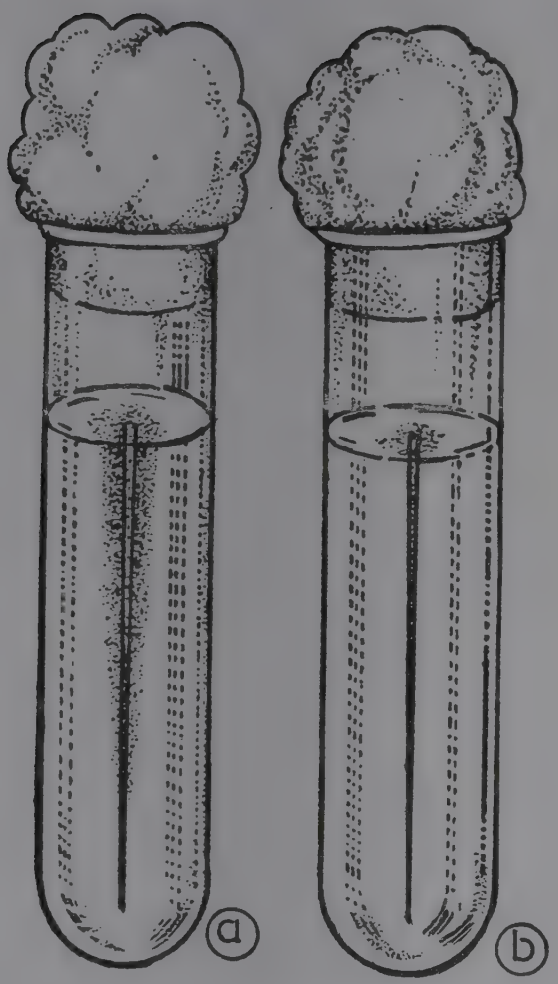

Fig. 40. Motility test in (a) *P. pseudotuberculosis,* (b) *P. pestis.*

green or methylene blue nor produce H_2S. In this respect all plague strains irrespective of their cultural and antigenic qualities behave in the same manner and these tests may be utilised as methods for differentiation between the two types of organisms.

Action on Carbohydrates

Using 19 sugars *e.g.*, glucose, galactose, levulose, arabinose, xylose, maltose, trehalose, mannitol, dextrose, salicin, rhamnose, saccharose, lactose, raffinose, inulin, dulcitol, sorbitol, inosite and isodulcitol, the author found the first 10 sugars to be fermented by *Y. pestis* producing only acid. Of these, galactose is fermented in 48-72 h, salicin in 72-96 h and the rest in 24 h. *Y. pseudotuberculosis* strains also ferments generally the same 10 sugars but the sugars that distinguish them from *Y. pestis* are rhamnose and isodulcitol which produce acid with the former and not with the latter. The plague strains isolated at Calcutta and Bombay do not ferment glycerol in contrast with *Y. pseudotuberculosis* which does so readily (Fig. 41). The reaction is speeded up by growth and fermentation of sugars using the following medium.

A simplified medium containing 1 per cent peptone, 1 per cent sugar and 0.2 mg of dried proteolysed liver at *p*H 7.4 has given better efficiency

Fig. 41. Glycerol fermentation test of virulent *Y. pestis* and *Y. pseudotuberculosis*.

of fermentation test with plague and pseudotuberculosis organisms as that of peptone water sugar medium containing 2 per cent rabbit serum (Seal, 1972 a).

Differential sugar fermentation reactions of the plague and pseudotuberculosis strains were carried out. Of the 30 laboratory maintained virulent and avirulent plague strains and 70 freshly isolated plague strains from human cases and rats tested, only two among the laboratory strains failed to ferment dextrin and caused late fermentation of maltose (4th and 6th day), whereas the avirulent protective Madagascar strain EV and the avirulent non-protective strain TRU failed to ferment galactose. On the whole all plague strains irrespective of virulence and protective qualities behave in the same manner in respect of sugar fermentation test.

To distinguish *Y. pseudotuberculosis* type strains from one another the three sugars—raffinose, trehalose and dextrin are useful. PR (I) ferments dextrin, PR (II) trehalose, PR (III) raffinose and PR (IV) raffinose and trehalose and *P. aviseptica* saccharose, inulin and sorbitol.

Chemical Antigenic Structure

It has been shown in the earlier Chapters that the author isolated two specific protein fractions $P\frac{1}{3}$ and $P\frac{1}{2}$-$P\frac{1}{3}$ by precipitation of the supernatant of Haffkine plague vaccine by Na_2SO_4 (33% and 50-33% saturation respectively) corresponding to antigen A specific for virulent and avirulent protective *Y. pestis* and antigen B ($P\frac{1}{2}$-$\frac{1}{3}$) fraction corresponding to the somatic antigen common between *Y. pestis* and *Y. pseudotuberculosis*. The culture filtrate of *Y. pseudotuberculosis* treated in a similar manner yielded fractions serologically reacting with antigen B or $P\frac{1}{2}$-$\frac{1}{3}$ fraction and not with antigen A or $P\frac{1}{3}$ fraction of *Y. pestis*.

A serologically active protein fraction corresponding to antigen A above has also been isolated from the water-soluble extracts of virulent *Y. pestis* grown on casein hydrolysate agar at 37°C for 72 h. This fraction identified as L/auto $P\frac{1}{3}$ reacts with an antiplague serum at a high dilution of 1280.000 and is completely absent in *Y. pseudotuberculosis*. This antigen being the most specific for *Y. pestis*, an antiserum prepared against this fraction agglutinates only *Y. pestis* and not *Y. pseudotuberculosis*. There is, however, one exception and that is the non-protective avirulent *Y. pestis* (generally a laboratory dissociant) which does not possess antigen A or water-extractble protein fraction (L/auto $P\frac{1}{3}$). It behaves virtually like *Y. pseudotuberculosis*. Such strains can be differentiated by serological tests given in Table 45.

Thus antigen A, specific for *Y. pestis*, is absent in *Y. pseudotuberculosis* strain. Furthermore water soluble extracted $P\frac{1}{2}$ fraction antiserum can only pick up *Y. pestis* virulent and avirulent *protective* and *Y. pseudotuberculosis*.

Serological Reactions

By means of agglutination, cross-agglutination, absorption and cross-absorption of agglutinins, precipitation and cross-precipitation tests the author (Seal, 1951 a) worked out the antigenic relationship of *Y. pestis* and *Y. pseudotuberculosis* as summarised in Table 35.

Certain Confirmatory Tests

1) *Precipitin reaction between the protein fractions of Y. pestis and Y. pseudotuberculosis*: By using two antisera one against $P\frac{1}{2}$ fraction of virulent *Y. pestis* and the other against $P\frac{1}{3}$ fraction of *Y. pseudotuberculosis* against the strain, the former will pick up *Y. pestis* and the latter *Y. pseudotuberculosis* but isolation of protein fraction requires time and experience.

2) *Animal protection test*: Guineapigs being susceptible to both types of organisms, if infection follows inoculation with unknown organism in these animals if protected or prevented from death by known high titre anti-*Y. pestis* serum or by anti-pseudotuberculosis serum the unknown organism would thus belong to the type of antiserum which has prevented or given protection against death due to the corresponding infection (Swiss mice may be used). The former test will be inconclusive if the organism has lost its virulence but not the latter except in case of gross antigenic deterioration.

Table 45. Serological relationship beween *Y. pestis* and *Y. pseudotuberculosis*

Antisera	Virulent or avirulent protective *Y. pestis*	Av. non-protective *Y. pestis*	*R. pseudo-tuberculosis*
1. Antiplague (virulent) antiserum	+	+	+
2. Antiplague (virulent) antiserum absorbed with *Y. pseudotuberculosis*	+	0	0
3. Antiserum against boiled (30 min) *Y. pestis*	0	+	+
4. Anti-pseudotuberculosis serum	0	+	+
5. Anti-pseudotuberculosis serum absorbed with *Y. pestis*	0	0	+
6. Anti-pseudotuberculosis serum absorbed with *Y. pestis* (boiled 30 min)	0	0	+
7. Anti-autolysate plague protein (L/auto $P\frac{1}{3}$) serum	+	0	0
8. Antiserum against antigen B of *Y. pestis*	0	+	+
9. *Y. pseudotuberculosis* $P\frac{1}{3}$ or $P\frac{1}{2}$ protein antiserum	0	+	+

CHAPTER XI

Diagnosis and Treatment of Plague

With the reappearance of plague in Calcutta in 1948 certain standard procedures for its diagnosis were considered to be of utmost importance.

In order to prevent the spread of the disease to new areas and adopt control measures as early as possible in those areas, need was felt for undertaking research projects for the diagnosis of positive and negative plague cases, for trial and utilisation of new drugs and diagnostic procedures and for compilation of incidence, fatality rate and other statistical data particularly those related to 'The International Intelligence Service'.

Moreover, the danger of mistaking plague for other diseases was of great national and international consequence. Some cases were diagnosed on clinical grounds alone and were given drug treatment before admission to hospital, creating further difficulties in early and correct diagnosis. A differential diagnostic system was, therefore, to be developed to identify plague from other diseases resembling it such as, septic bubo, certain venereal buboes, tubercular or filarial adenitis, parotitis (in which sulphadiazine helps in quick subsidence) and common broncho-pneumonia cases. Field workers were, therefore, advised to undertake gland puncture and collect the clinical materials before administering streptomycin and sulphadiazine.

For arriving at a correct diagnosis and effective treatment the following steps were recommended:

1. Collection of epidemiological data on a specially designed schedule by house to house visit.
2. Thorough clinical examination with particular attention to the sets of glands affected and their nature.
3. Differential count of blood in certain cases, and culture of blood and bubo materials.
4. Collection of sera from patients for serological examination.
5. Animal inoculation test in a few instances only.
6. Collection of dead and live rats in the houses or nearby areas and examining them for physical evidence of plague by dissection and by examination of spleen smear and culture of splenic material and blood from heart. This would provide evidence of area of infection and possible involvement of human beings.

7. Distribution of the classical features of four clinical varieties of the disease namely, a) pestis minor, b) bubonic, c) septicaemic and d) pneumonic, to the field workers as well as to the hospital staff. Excepting pneumonic type one clinical type might merge into another and hence the field workers were instructed to look for the following common features:

Incubation Period

The incubation period is of great importance to the epidemiologist for the detection of source, conncetion of cases and even for diagnosis. It varies from 2 to 10 days (generally 2-6 days) and is mostly less than 3 days. It may be different for different epidemics and even for different stages of the same epidemic, and may be shortened at the height of the epidemic. It is normally shortest in case of pneumonic plague (one to three days).

Onset

It is generally sudden short and insignificant without any prodromal symptoms. The patient may feel a little unwell and chilly or giddy, may have headache, mental dullness and pain or discomfort at the site of the coming bubo. Temperature may rise from 100-104°F and P/R-120/30 or 40. Tongue may be furred vomiting and/or diarrhoea may ensue. Patient staggers like a drunken man if asked to walk.

Course

Patient has an anxious lock with flushed skin bright blood shot and staring eyes and dilated nostrils. Temperature becomes fluctuating. It may rise on the first day, come down on the 2nd or 3rd day, again ascend on the 4th day and fall by lysis on the 7th or 8th day. It may, however, fall temporarily to rise for the last time before death. Bleeding from nose (epistaxis), stomach or intestines (haematemesis and melena) and bladder (haematuria) is not uncommon.

Pulse

The pulse is rapid (120-180 per min), at first full or of moderate tension, later weak and intermittent. It may finally become so rapid and imperceptible that it can no longer be counted. Sudden death from heart failure may take place.

Blood

Coagulability of blood is diminished. Polymorphonuclear leucocytes may be 30-100,000 per cmm and erythrocytes may increase to 6 million per cmm. Bacteraemia may be detected in about 30 per cent cases.

Skin

Skin is hot and dry. Erythema and even macular rashes may be present. Eruptions of various types have been noted: vesicles, pustules, haemorrhages

(petechial or ecchymotic). A blister phlyctenule may be present at the site of the flea bite from whence the lymphatics drain into the lymph gland causing bubo (2 cases were seen in the present outbreak; (Fig. 42).

Urine

The specific gravity of urine increases but its volume is diminished. Plague bacilli are often excreted in urine. Traces of albumin and blood may be present. There may be anuria before death.

Abdominal Symptoms

Patient feels thirsty. Tongue is thickly coated with white fur, teeth and lips filled with sordes. Vomiting and diarrhoea and even constipation may be present. Abdomen may be tender and liver and spleen moderately enlarged and even tender on deep palpation if heart failure starts.

Lung Symptoms

Apart from pneumonic type, lung symptoms are invariably present in severely affected patients, particularly in septicaemic type though the symptoms and signs are not commensurate with the actual lung condition. In the terminal phase, however, lung oedema leading to dyspnoea and cyanosis is almost always present.

Mental Symptoms

Patient may be apathetic weak or delirious. Cerebration and speech control are often impaired, memory may be confused and patient may become violent and try to rush away from bed especially in pneumonic plague. Nervous excitement is less conspicuous in female patients, but children may have convulsion.

Termination

Patient may be extremely prostrate, may develop air hunger, and may die of sudden heart failure. In a few cases there may be marked agony accompanying oedema. Low muttering delirium may develop into coma to end in death which usually takes place within 3 to 5 days.

Recovery

In favourable cases temperature falls by lysis, tongue becomes moist and clean, pulse rate diminishes, and convalescence starts. Even now death may ensure from cardiac failure, septic infection, suppuration or secondary haemorrhage.

General Features of Different Clinical Types of Plague

Mild Type of Plague (Ambulatory or Pestis Minor)

This type is generally encountered either at the beginning or end of an

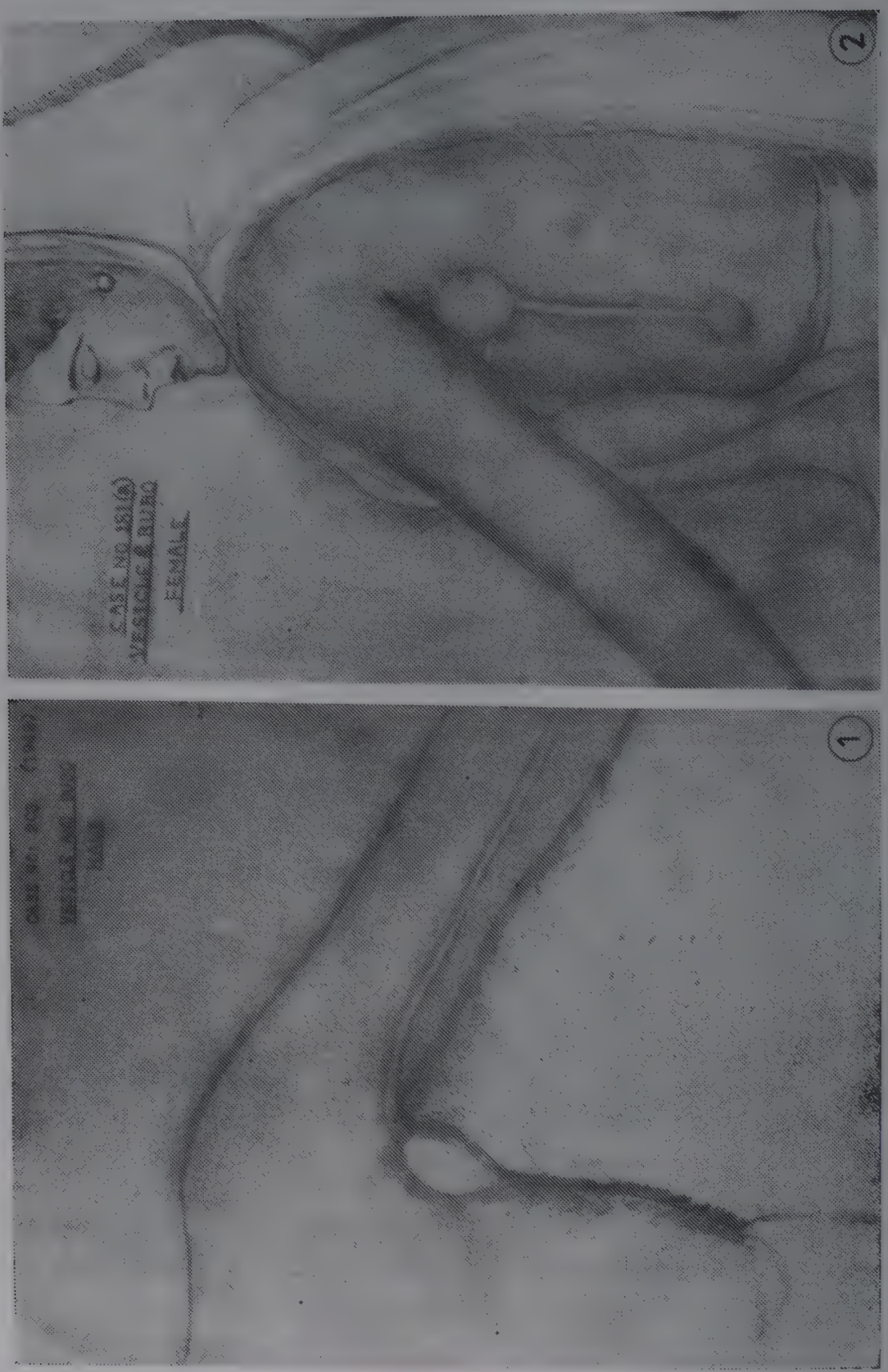

Fig. 42. Phlyctenule with enlargement of gland in (1) a man; and (2) a woman.

epidemic. Patient has little or irregular fever. Slight tenderness of the affected gland or possibly suppuration. The patient may not seek relief; diagnosis by bubo puncture and animal inoculation.

Bubonic Plague

Apart from general symptoms painful swelling appears in groin, axilla or side of the neck, elbow, bicipetal groove, back of the neck and popliteal space. Bubo is usually unilateral but may be bilateral and even multiple. Its localisation perhaps depends upon the habits of wearing clothings and coverings and manner of sleeping (floor, cot, *etc.*). The skin over the bubo is hot and red, painful and tender to touch, and feels boggy and elastic. If incised, it discharges yellow fluid, characteristic of plague bubo. With pus formation the bacilli disappear from the gland. It undergoes slow resolution. Occassionally softening and ulceration may occur and in some instances gangrene.

Septicaemic Plague

Thirty per cent of bubonic cases may lead to bacteraemic stage when bacilli are recoverable from blood. Septicaemia starts with overwhelming infection and weak defence mechanism. Some of these cases die (in 3 or 4 days) even before the appearance of bubo. In certain septicaemic cases diarrhoea with blood, and haemorrhage under the skin may occur. Leucopenia is the rule in these cases. Plague bacilli may be seen in direct blood smear. Such cases may appear at the early stage of the epidemic and are generally likely to be missed. Lungs may be affected but the patient usually succumbs before bacilli appear in sputum.

Pneumonic Plague

1) Bubonic cases may lead from septicaemic to pneumonic type through lung involvement and then infection may spread to fresh cases through droplet or,

2) The spread of infection takes place from pneumonic plague cases to fresh cases directly through droplet.

At the onset the patient feels suddenly ill with rise in temperature and suffers from general symptoms enumerated earlier. Temperature may decline below normal towards the end. P/Ris 110-130/130-50. The face expression of the patient is anxious, with conjunctive injected face flushed and bloched. There is extreme prostration and clouding of consciousness, delirium, restlessness and tendency to run out of bed; later, face assumes dark and dusky hue and cyanosis appears.

The patient complains of pain with restricted feeling in the chest followed by soft and hacking cough and later dyspnoea (air hunger and breathlessness). Sputum at first is scanty (with mucus only), later it is abundant and blood-tinged, typical of croupous pneumonia. Still later it becomes frothy and of bright red colour with discharge of enormous numbers of plague bacilli.

Towards the end patient becomes dyspnoeic and shows signs of extreme exhaustion and collapse. In most instances mind remains clear till the end. Cyanosis is common and ecchymosis may appear in different parts of the body. Physical signs in the lungs are not commensurate with the pathologic state in the lungs except that localised areas of dullness may be distinguished. Rales are not heard except before death or when oedema starts. Respiratory sounds are usually feeble or tubular and in a few instances dry pleuritic rub may be present. The spleen is usually not palpable and lymphatic glands are not enlarged. Bloody diarrhoea with discharge of plague bacilli may occur. Leucocytosis may be absent in blood. The heart is usually dilated on the right side. The second pulmonary sound may be accentuated but soon lost. In advanced cases cardiac involvement is marked, pulse becomes rapid, soft and finally cannot be felt. The disease is rapidly fatal, death taking place after 2 or 3 days and rarely 4 days.

Atypical Types of Plague

Several types of atypical cases have been described in the literature particularly in connection with pneumonic plague. These are, 1) tonsillar plague, 2) anginose plague (sore throat), 3) general eruption, 4) plague-pox (variolous plague), 5) subpectoral bubo, 6) plague meningitis, 7) atypical pneumonia (subacute pneumonia). These cases are rare but likely to be missed.

Black Death

Mention may be made here of 'black death' which menaced the European continent in the middle ages. It was characterised by black spots and tumours in armpit and groin complicated further by haemorrhagic manifestation or carbuncle in the skin ending in death, which made it known as black death. Once it had taken hold it could develop into a pneumonic form which needed no rats or fleas to transmit the infection but spread simply from the tainted breath of those already stricken. Carbuncles and necrotic patches could develop even in bubonic cases usually as a result of metastatic infection through blood stream. Though rare now, such skin manifestations seem to have been common occurrence during the historical plague outbreaks, particularly the black death and great plague of London in 1665.

Meningeal Involvement

According to Meyer *et al* (1937) instances of plague meningitis or rarely encephalomyelitis were observed by some of the early workers such as, Austrian (1895) and German Plague Commission (1899).

Diagnosis of Plague

It depends upon (a) clincal signs and symptoms; and (b) laboratory methods. In regard to the clinical signs and symptoms it is to be remembered

that the patient does not often mention abobut bubo. Similarly in a community which did not have plague for some time, cases are generally overlooked. Therefore, for the diagnosis pneumonic plague attention may first be drawn to the cases among doctors or nurses attending such cases.

Laboratory Methods

i) *Bubo puncture*: It is done with a sterilc hypodermic syringe containing a drop of saline broth. The material recovered is cultured on 5 per cent rabbit blood agar, nutrient broth or casein hydrolysate broth, a smear is made on glass slides and a portion is injected subcutaneously or intraperitoneally into a white mouse. The smear is stained by both Gram and Leishman or carbol-thionin stain.

ii) *Blood examination*: Direct smear examination may show bacilli in septicaemic cases. Total WBC and differential count, culture examination in 5 per cent rabbit blood agar, nutrient broth or casein hydrolysate broth and mouse or guineapig inoculation should be done for detecting plague cases.

iii) *Sputum examination* (in case of pneumonic plague): Staining by methylene blue and thionin and Gram stain may be done to look for bipolar organisms.

iv) Post-mortem examination of spleen and liver may indicate plague infection, if any.

Difficulties in Bacteriological Diagnosis

1) In bubo puncture the technique may be defective.

2) The organism may not be present or somehow missed in the first puncture.

3) If sulpha drugs or streptomycin are applied prior to admission, stages of the organism seen are actually degenerated.

4) The isolation of organism depends on culture technique used.

5) Identification from sputum or throat sore is very difficult but the bipolar character becomes evident in specimens examined after culture.

B. *Differential Diagnosis*

i) *Differential diagnosis of one form of plague from another*: Both septicaemic and pneumonic form may develop from bubonic form and buboes may develop in septicaemic and even in pneumonic form but at a little late stage. So difficulty may arise when the cases are seen at the late stage. Again, the presence of plague bacilli in the sputum does not always mean pneumonic plague. The bacilli may appear in case of tonsillar plague or secondary lung involvement or lung oedema also.

ii) *Differential diagnosis of plague from other infectious diseases*: The following diseases have been mistaken for plague and vice-versa—adenitis (venereal, coccal, tubercular, mumps, lymphogranuloma, climatic bubo, glandular fever), influenzal pneumonia, typhoid fever and typhus fever occurring in plague season. Malignant malaria, relapsing fever and dengue may resemble

septicaemic plague. Even measles, chickenpox, smallpox, meningitis and appendicitis with inguinal bubo, tuberculosis with haemoptysis and high fever, broncho-pneumonia and bronchitis, tularemia and croupous pneumonia have been mistaken for plague.

C) Serological Tests

i) Agglutination test with sera of convalescent patients can now be done by the live suspension of plague bacilli obtained from casein hydrolysate broth or agar culture as defined by the author earlier.

ii) Complement-fixation test with patients' serum by using specific soluble plague proteins isolated by the author.

Treatment of Plague

Prior to 1941 the only treatment of plague worth mentioning was the antiplague serum originally prepared by Yersin (1897) and perhaps the iodine solution as the only available chemotherapy. The use of sulphanilamide around 1940 generally improved the prospect of curing bubonic plague. During the decade 1940-50 most outstanding success was achieved in the treatment of all forms of the disease including pneumonic plague by means of antibiotics, particularly streptomycin. These therapies reduced the fatality rate from 80-90 to 8-16 per cent or even less. Since the epidemic of plague in India practically ceased after 1963-64 and fatality rate was greatly reduced, very little change or addition to the methods of treatment was considered necessary.

Serotherapy

In the absence of any specific drug for the treatment of plague in the earlier part of this century the only useful remedy was serotherapy. It was effective in treating milder form of bubonic cases but the results were poor in septicaemic cases and disappointing in pneumonic cases except for some occasional success reported by some workers (Wu Lien Teh, 1926; De Moura *et al*, 1931; Joltrain, 1936). Sokhey (1936) and Sokhey and Dikshit (1940) claimed 83.0 per cent cure rate in experimental plague in mice, the mouse-protective dose of this Haffkine Institute antiplague serum being 0.05 ml against 0.03 ml of antiserum produced in buffalo by Naidu and Mackie (1931). With E.V. Madagascar antiplague serum Girard (1941b) and Le Gall (1943) claimed about 60–65 per cent cure rate against Sokhey's (1941-48) overall cure rate of 76.5 per cent in 157 cases treated with antiplague serum only but with 71 bacteraemic cases the rate of cure was only 49.3 per cent.

The poor quality of antiplague serum was due to the use of the then available rough strain cultured in unsuitable medium. This difficulty was overcome by the author (Seal 1941, 1943) following the introduction of protein-free casein hydrolysate broth as the culture medium and isolation of specific soluble antigen in pure form. There was appreciable improvement in antiplague serum production in horses and in estimating its titre (Seal, 1953).

According to Meyer *et al* (1952) the pooled experience of the latest use of anti-plague serum in the Eastern hemisphere gave a fatality rate of 45.13 per cent in 3840 treated cases against 83.5 per cent in 1726 untreated cases. In the Western hemisphere the fatality rate in 19,540 treated cases was 22.75 per cent against 74.07 per cent in 213 untreated cases. During this time the Hooper Foundation of San Fransisco also carried out a comparative trial of antisera produced in horses, rabbits and guineapigs on mice and found that the antisera produced in rabbits were superior to others but to obtain the desired results the serotherapy should be started as early as possible. Better results were obtained with antiserum particularly when produced by using specific soluble protein antigen isolated by the author (Seal, 1943, 1953). The titre of the antiserum could easily be estimated by quantitative agglutinin and/or precipitate N test. But by the time this was achieved very effective sulpha drugs and antibiotics were discovered and the use of serotherapy became almost redundant although Sokhey considered that the combined sero- and chemotherapy would be preferable in septicaemic and pneumonic cases (Sokhey and Wagle, 1946). The results are summarised in Table 46.

Sulphonamides

The most important factor that should be considered in the evaluation of the results of treatment is the degree of septicaemia and the clinical conditions of the patient (Meyer *et al* 1952; Sokhey and Wagle, 1946). Evaluation should also depend upon the dose of the drug, its methods of administration and supervision of the treatment. The required concentration of the drug in the blood is 5 to 10 mg/dl of blood (Wagle 1948; Macchiavello, 1949). A higher concentration of sulpha drugs *i.e.*, 10-20 mg/dl of blood was advocated by Sokhey *et al* (1952), the first dose of 2 g being given iv.

The earliest compound tried was prontosil (p-amino-phenylsulphomanide; Vine, 1928; Caman, 1938) followed by sulphanilamide by Van Hoff (1938) and sulphapyridine which was found to be better than sulphanilamide (Durand, 1929; Schutze, 1939; Girard and Girard, 1939). Soon sulphathiazole was discovered and synthesised at the Haffkine Institute, followed

Table 46. Treatment of septicaemic and other plague cases with combined sero and chemotherapy

Drug or drug + antiserum	No. treated of septicaemic cases			All cases
	Mild	Severe	All	
Anti-plague serum	29 (75.8)	42 (38.0)	71 (49.3)	157 (76.5)
Sulphathiazole	27 (85.2)	104 (50.1)	131 (58.0)	345 (77.1)
Antiplague serum + sulphathiazole	4 (100.0)	21 (69.1)	25 (68.0)	60 (80.1)

Figure in parentheses are the percentage cure rate.

successively by sulphadiazine, sulphamerazine and sulphamezatine, all being used for the treatment of plague by turn. The results of treatment by sulpha drugs have been reviewed by Pollitzer (1954). In a series of 786 cases treated between 1948 and 1953 the cure rate varied between 80 to 100 per cent. The combined experience of Sokhey and Wagle (1946) in six field trials on all plague cases and also separately in septicaemic cases is summarized in Table 47.

On the basis of above results sulpha drugs can be arranged in the following order of increasing efficiency: Sulphapyridine, sulphathiazole, sulphamerazine and sulphadiazine, the last drug having been found to be a little superior to sulphamerazine in septicaemic cases. The combined sulphathiazole and anti-serum treatment proved to be the next best. Sokhey and Wagle (1946) found anti-plague serum to be even better than sulphapyridine in septicaemic cases (Table 48).

The combined results of treatment by all Indian workers revealed a survival rate of 68 per cent in cases treated with sulphanilamide, 45.4 per cent in cases treated with sulphapyridine, 79.5 per cent in cases treated with sulphathiazole, and 91.4 per cent in those treated with sulphadiazine. The highest survival rate (91.7%) was recorded in cases treated with sulphamerazine. Barring sulphamezathine which was used only in a few cases, sulphamerazine seemed to be even superior to sulphadiazine (chi^2 test being 4.7896 for one degree of freedom). But Sokhey and Wagle (1946) found sulphadiazine superior in septicaemic cases and the Calcutta workers complained of kidney complications with sulphamerazine. Accordingly sulphadiazine has remained the drug of choice and if administered early, satisfactory results can be expected. Meyer *et al*'s (1952) work also supports this.

Table 47. Treatment of all cases of plague with different sulpha drugs and antisera

Drugs/antisera	No. of cases treated	No. survived
Anti-plague serum (Haffkine Institute)	167	127 (76.05)
Sulphapyridine	122	89 (73.0)
Sulphathiazole	345	266 (77.1)
Sulphathiazole and antiserum	60	48 (80.0)
Sulphadiazine	198	182 (91.2)
Sulphamerazine	149	145 (97.5)
Iodine solution	149	69 (46.3)

Figures in parentheses represent the per cent survived.

Doses

The following doses of the different sulpha drugs were recommended:

Drug	*Doses*	*Remarks*
Sulphapyridine and Sulphathiazole	10-14 g on first day 6 g on subsequent days up to 10 days depending upon severity.	The drug can be given intra-muscularly or iv if needed.
Sulphadiazine	4 g initial dose followed by 2 g every 4 h later. Thereafter 1 g every 4 h till the patient's temperature remains normal for 2 days.	Drug concentration 10-20 mg/dl of blood.
Sulphamerazine	4 g initial dose, followed by 2 g 4 hourly. Thereafter 1 g 8 hourly till temperature remains normal for two days.	—As above—
Sulphamezathine (reported by Dutta Gupta, 1948)	3 g by iv route, then 2 g 4 hourly for 4-7 days orally.	

N.B.: Higher initial dose is necessary for septicaemic and pneumonic cases.

Antibiotic Treatment

With regard to the treatment of primary pneumonic cases reviewed by Pollitzer (1954), as many as 13 investigators reported cure in 24 out of 25 cases by streptomycin alone, whereas 9 other cases were successfully treated by streptomycin combined with sulpha drugs and sometimes in conjunction with penicillin and antiplague serum. The only case that died had a lung abscess which was not due to plague bacilli. In this connection, Girard (1952) pointed out the necessity of using sulphonamides or penicillin; an adjuvant treatment in such cases. The last Expert Committee of WHO therefore recommended that 'in pneumonic plague cases administration of sulphonamides or broad spectrum antibiotics might be necessary in order to prevent secondary infections or their sequelae'. This committee also recommended that such dose should be selected as will maintain the concentration of the antibiotic or the drug at a minimum level of 5-10 mg/dl. Accordingly, the minimum dose in any case should not be less than 0.5 g im, to be repeated 4 hourly or at longer intervals if the case is mild, and at shorter intervals of 3 h according to the severity of the case. In case of moderate severity, a dose of 2 g per day may be the minimum and for frankly pneumonic cases 2.3-3 g dose per day may be maintained until the case is considered safe enough for relapse or other complications.

In the case of pneumonic plague sulphadiazine alone might be enough. In bubonic-septicaemic a few initial doses of streptomycin may be followed by sulphadiazine to complete the treatment.

Table 48. Treatment of septicaemic plague cases by different drugs and antiserum

Drugs/antiserum	Mild septicaemic cases (1-10 organisms/ml of blood)		Severe septicaemic cases (> 10 organisms/0.25 ml of blood)		All septicaemic cases	
	Cases	Survivors	Cases	Survivors	Cases	Survivors
Anti-plague serum	29	22 (75.8)	42	16 (38.0)	71	35 (49.3)
Sulphapyridine	26	21 (80.8)	36	10 (27.8)	62	25 (40.4)
Sulpeathiazole	27	23 (85.2)	104	53 (50.1)	131	76 (58.0)
Sulphathiazole and antiserum	4	4 (100.0)	21	13 (61.9)	25	17 (68.0)
Sulphadiazine	—	—	68	48 (70.1)	61	48 (78.7)
Sulphamerazine	—	—	22	15 (68.2)	22	15 (68.2)
Iodine	19	5 (20.3)	56	2 (3.6)	75	7 (9.3)

Figures in parentheses represent the percentage survived cases.

In 1952 Girard, and Mercior and Maccoumb separately reported successful results with another antibiotic-chloramphenicol. The latter also treated 5 cases of pneumonic plague with terramycin but according to Pollitzer (1954) antibiotic should be used only where specially indicated. The tetracycline is another drug preferred by some workers given usually in large doses (4-6 g daily) during first 48 h. Intravenous therapy during first 24 h is essential for severely ill patients, but should be supplemented by oral administration if the patient tolerates it.

Combination of Sero and Chemo or Antibiotic Thereapy

Pollitzer (1954) considered the use of antiplague serum in addition to antibiotics, in special circumstances. In author's view a combination of sulphadiazine and antiplague serum would be an ideal method, particularly in septicaemic cases and in certain selected cases, and streptomycin and in certain selected cases, and streptomycin and antiplague serum in primary pneumonic cases. Combined administration of sulphonamides and antiplague serum was observed to give better results than either of them alone, particularly when there was no antitoxic action of sulphonamides (Sokhey, 1936; Schutze, 1939; Girard and Girard 1939; Meyer 1950b; Seal 1960c).

Management

These have been considered by Pollitzer as follows: (1) rest in bed, (2) diet and fluid intake; (3) supporting treatment and (4) treatment of buboes and complications. While the clinicians will use their discretion in every individual case, it may, however, be emphasised that particular care be taken to give the patient complete bed rest.

Prophylaxis

The author obtained very good results in a heavily infected area where vaccination against plague had been started by giving the exposed person sulphadiazine in doses 2 g for the first 3–5 days in addition to two doses of vaccine. During epidemics many people escape the second dose either by chance or wilfully. This has been found to take care off the incubatory cases which were not uncommon during the initial stage of the Calcutta epidemic and of the vaccine drive. For the same reason, the plague workers, even though they may have been protected by vaccination against plague infection should fortify themselves with sulpha drugs at the commencement of the epidemic, or at the initial stage of their work in the heaviy infected areas and at the time of attending pneumonic plague cases.

Since the advent of the effective sulpha drugs seroprophylaxis has beeu out of practice. Even the contacts of pneumonic cases are taken care of by the sulphadiazine prophylaxis. Even so, it would be desirable to use seroprophylaxis in cases of very close contacts of primary pneumonia cases, to whom potent antiplague serum (50 ml in case of adult and 25 ml in the case of children) may be given along with sulphadiazine or sulphamerazine.

Clerk and Goldberg (1943) (quoted by Pollitzer, 1954) gave in addition to plague vaccine serum doses (50 ml for adults and 25 ml for children) to 35 persons who had been in close contact with pneumonic plague patients. Only three of the serum treated contacts developed the disease and one of them was cured through administration of further doses of 400–500 ml of antiplague serum. It is noteworthy that (a) a woman who had been given 100 ml of anti-plague serum because she had attended 4 pneumonic patients, remained well; (b) three children who had slept with their mother up to and including the day she died from pneumonic plague and each of whom received 50 ml of specific anti-serum also did not develop the disease.

Pollitzer (1949) however, pointed out that it would not be advisable to make large scale use of prophylactic sulphonamide treatment when dealing with bubonic outbreaks because during these the danger of human infection is less limited as to space and time than in the case of pneumonic type where familiar explosions are the rule. However, one should not hesitate to use this method for the purpose of warding off bubonic plague attacks if special conditions such as the occurrence of laboratory or post-mortem accident or the occurrence of plague in a ship or port hitherto free from plague warrant this. Similarly, to administer antibiotics instead of sulphonamides would be inadvisable to avoid drug resistance in strains.

CHAPTER XII

Control and Prevention of Plague

The various methods used for the control of plague are:

1. Antirodent measures against commensal rodents *viz.*, killing by mechanical means, use of predatory animals, trapping, fumigation, poisoning and baiting and other methods.
2. Rat proofing of houses, godown *etc.*
3. Control of wild rodents.
4. Vector control by DDT insufflation, benzene hexachloride application, use of other insecticides and fumigation.
5. Direct control of bubonic plague through hospitalisation of patients, management of contacts, the evacuation, mass vaccination and chemo- and sero-prophylaxis.
6. Control of pneumonic plague by case detection, isolation and treatment of patients, management of contacts, protection of staff and disinfection of contaminated objects.
7. Disposal of the dead.
8. Control of the spread of plague at a distance through quarantine laws and international intelligence system.

Until 1947, the routine anti-plague measures in the different states of India consisted of cyanogas (calcium cyanide) fumigation of rat burrows, total cyanogas fumigation of the infected and the surrounding houses and occasionally of houses or villages during an actual epidemic outbreak, and single dose inoculation of the population by Haffkine plague vaccine.

Mass destruction of rats by baiting and trapping was undertaken as an inter-epidemic measure in certain states, while in a few towns such as, Bijapur and Pandharpur in Bombay state and Ootacamund, Coonoor and Kotagiri in Madras state, systematic cyanogas fumigation of burrows, grain godowns, and market places was done during inter-epidemic periods or as a routine every 3 or 4 months. Rat proofing of dwelling houses, godowns and other places did not receive adequate attention in India except perhaps in very big cities such as Bombay, Calcutta and Madras. In dealing with the control measures a greater stress should be laid on the above measures.

1. Antirodent Measures

A) Killing by Mechanical Means

The idea of spread of infection, especially that of plague by rats is very old in India and rats were killed not only for preventing spread of disease but also for saving destruction of food and clothing *etc.* Two methods were generally used: killing by clubs, sticks *etc.* and by traps. But killing rats by people during an outbreak of plague cannot be encouraged because killing of such rodents will rather facilitate spread of infection through infected fleas. In fact, during plague dead rats should not be touched by hands. They should be handled only after pouring kerosene over their dead body and the use of tongs. Some people keep cats, dogs, ferrets and mongoose for killing and driving away rats.

B) Trapping

Two types of traps are used namely, enclosing traps and killlng traps. There is still a third type of 'arresting' trap which traps rats without killing them and is used in USA. There are various patterns of enclosing traps, the main principle being provided with a trap door for entering and preventing exit. It is generally of wirecage type, $3'' \times 6'' \times 5\frac{1}{2}''$ made of strong wire in which as many as a dozen rats are sometimes caught. Traps are baited with some food (bread smeared with coconut oil or any other common attractive food). The 'arresting' steel traps are not baited but have a flat-form trigger and two steel jaws which are snapped together by means of a single flat spring

It is, however, important to keep the traps in perfect working condition. The technique recommended by Wiley (1945) for cleaning steel traps may be followed. Unclean traps with blood or body parts of previously caught rats will scare them away. Sometimes rats caught in traps escape by breaking the weakened traps. These should be placed with proper baiting at suitable situations by selection from the trappers own experience. However, trapping is essential for all types of plague control work.

C) Poisoning

The various types of poison baits that can be mixed with rat meals are:

i) *Arsenic trioxide*—It is highly poisonous and therefore, dangerous to man as well as domestic animals. The chances can, however, be reduced somewhat by adding tartar emetic in a ratio of 6 oz of tartar emetic to 1 lb arsenic oxide. Sodium arsenite (10%) is also as effective as arsenic trioxide and may be used.

ii) *Barium carbonate*—It can be used in proportion of 20 per cent by weight. It has been realized that poisoning if it is to be done must be both intensive and extensive *i.e*, not only the focal areas should be involved but also the neighbouring residences, godowns out-houses, garbage dumps and sewers (vertical system of rodent control). This should follow an initial rat

and household survey consisting of discovery of rat runs and sewers, collection of fresh droppings and watching fresh gnawings.

The choice of rodenticide depends on different variable factors, particularly the species of commensal rodents present, nature of the sites, attitude of the people and available funds and facilities. Similarly the choice of the bait bases and bait preparations is also of equal importance. Pre-baiting operations are also helpful in the use of poison baits on a wide scale. Successful pre-baiting ensures acceptance of the bait by rats (Barnett, 1947, 1948).

iii) *Other poisons used for baiting*—A brief statement is given below of other poison baits used with their overall results.

1. Phosphorus containing baits are limitedly used in foodstuff (not preferred now).

2. Red squill-obtained from red variety of *Urginea maritima* (available in countries around Mediterranean) is the oldest rodenticide. It has an LD_{50} of 500 mg/kg in wild Norway rats, and is not suitable for mice and *R. rattus*. One of the safest rodenticides, it should not have access to children and domestic animals.

3. Strychnine sulphate or strychnine alkaloid is poorly accepted by commensal rats; generally used for control of commensal mice and certain wild rodents. The following mixture is useful 28 g powdered strychnine alkaloid + 56 g common borax + 3.6 kg large flaked rolled oats distributed in tea spoonfuls or exhibited in little boxes.

4. Thallium sulphate (Th_2SO_4; heavy white crystalline powder fairly soluble in water) works well against *R. rattus*, LD_{50} being 35 mg per kg body weight, and is slow in killing. It is most dangerous for man and animals (domestic), the lethal dose for men being 20 mg/kg body weight.

5. Zinc phosphate ($Zn_3P_2O_4$; black powder slightly soluble in water, odour is attractive to rodents) considered efficacious for rats and mice, it is supposed to be more effective than arsenic trioxide or red squill and less effective than sodium fluoracetate and thallium sulphate.

6. Alpha naphthyl thiourea (known under the name ANTU) exerts a particularly fatal action on adult Norway rats. Its LD_{50} varies from 6 to 8 mg per kg of body weight. But both *R. rattus* and commensal mice were found to be far more resistant than the Norway rats.

7. Sodium fluoracetate (often called 1080, a light white crystalline compound, tasteless with no marked odour) kills rats and mice within 1–8 h and is poisonous to domestic animals. According to workers in USA, it should be given in water; 7 g of 1080 per gallon of water is sufficient to kill *R.rattus* and mice.

8. Anticoagulants (Warfarin; a stable colourless crystalline solid, free from odour and taste. Bait is prepared in rolled oats). It is used at a concentration of 0.05 mg per gram of the bait material and effective for the control of *R. norvegicus*. A little more concentration is necessary for *R. rattus*.

D) Fumigation

This is the commonest method now-a-days used in India for the destruction of rats. Calcium cyanide [cyanogas $Ca(CN)_2$] is generally used. Other gases which may also be used are carbon disulphide, carbon dioxide, carbon monoxide, chlor-picrin and hydrocyanic acid as well as carbon dioxide snow. It merely suffocates the rodents. On the other hand, CS_2 is too dangerous to handle. Its vapour have an unpleasant smell and it is extremely inflammable and apt to be explosive when mixed with air. Its main use is rather as an insecticide. It has been used as rodenticide in California, South Africa and South-East Russia.

Carbon monoxide is generally used in combination with CO_2. Another gas-chlorpicrin (trichloro-nitromethane—CCl_3. NO_2) is also an insecticide being occasionally used for rat destruction (Lewis *et al* 1945). It is supposed to be superior to other fumigants as it is free from fire and explosion hazards.

i) *Hydrocyanic acid* (*HCN*): It is a colourless liquid in the pure state below the boiling point of 26°C. It is one of the most rapidly acting fatal poisons known, and hence the workers supplying HCN are to be protected with gas masks. Applied at high concentration it is harmful to vegetables. In the opinion of Williams (1931) even when protected by gas masks one should not remain longer than 5 min in the atmosphere containing concentration of the gas amounting to 2 oz per 1000 cu.ft. (2 g/m^3). Again, articles exposed to the gas should be aired sufficiently long to rid them from gas, particularly if the weather is cold and moist, because water quickly absorbs the gas and holds it. A room containing articles and food materials should be aired for 24 h before using the food materials.

Hydrocyanic gas can be used for rodent as well as insect control. It is generally used for fumigation of ships and disinfestation of warehouses and mills and is not used for antiplague work in general. For that purpose calcium cyanide CA $(CN)_2$ known as cyanogas is widely used.

ii) *Cyanogas*: It is available in the from of a fine greyish white powder for pumping operation. One commercial brand is available in the form of tablets together with a special pump which first grinds the tablets and then blows out the resulting fine powder. Three brands of the preparation are available in the market producing HCN on the basis of chemical reaction [$Ca(CN)_2 + H_2O$ (from moist air)-$2HCN + Ca(OH)_2$]. A brand (42.30% $Ca(CN_2)$ produces 23.31 per cent HCN, B brand (84.94% $Ca(CN)_2$) produces 46.80 per cent HCN and C brand (with 35.90% $Ca(CN)_2$) produces 19.78 per cent HCN.

Sokhey *et al* (1939) found maximum yield of HCN by 'A' brand of the preparation in 30 min and in 'B' in 15 min while 'C' took about 90 min to reach even this low concentration. It is now used mainly for treatment of rat burrows which after pumping in fine dust should be promptly blocked. Careful inspection of the ramifications of rat burrows has to be done and mouths of those ramifications closed before pumping. A mixture of Port-

land cement, sand and broken glass should be used for sealing burrows in lime mortar floors and good rubble walls. Holes in mud floors and poorly built walls may be sealed with moistened clay and with pieces of sod. Workers in India found that one pound (0.46 kg) of calcium cyanide was sufficient to treat about 60 average burrows or 100 small burrows. Small enclosed spaces inside buildings, such as the boxes in bases of shelving, counters and lockers may be spot fumigated if there is no danger of the dust going into living quarters or other occupied rooms. Thronton (1930) prescribed the following procedure for fumigating huts or small houses.

(i) Remove all occupants (man, animal and foodstuffs); (ii) dust all rodent burrows; (iii) hang all clothes, beddings and similar articles on rafters; (iv) spray floor lightly with kerosene emulsions; (v) cover huts completely with canvas sheets; (vi) pump calcium cyanide into rooms and thatched roof using $1\frac{1}{2}$ to 2 pounds per 1000 cubic feet (25–35 g/m^3) holding pump in inverted position to do this quickly; (vii) hold huts under fumigation for at least 4 h; (viii) remove canvas, open doors and windows, wait a little before entering; (ix) air clothing and beddings *etc.* in the open, and (x) sweep floor before using the hut next morning, preferably keep occupants away till next morning.

Any case of cyanide poisoning must be treated rapidly by means of amyl nitrite ampoules, sodium nitrite solution or sodium thiosulphate solution iv in a hospital. Artificial respiration is to be given if breathing stops. It has to be understood that the reductions of rats and fleas affected through cyanogassing operation are temporary, so repeated operations are necessary, as will be seen from the results of fumigation procedure in villages by cyanogas shown in Tables 49 and 50.

(iii) *Methyl bromide* (CH_3 Br.): It is a colourless gas at ordinary temperature but is heavier than air. It is supplied in cylinders and has been used in Great Britain to deal with considerable mouse infestations in hangers where bagged grains were stored. Workers had to wear masks.

(iv) *Sulphur dioxide* (SO_2): It is generated by combustion of sulphur in pots or with the aid of a special apparatus called Clayton machine. It has been

Table 49. Cyanogas fumigation after rat-fall

	Year of operation	
	1948	1949
No. of villages under treatment	—	468
No. of villages fumigated after rat-fall	35	18
No. of villages reporting human cases after fumigation	16	11*
Total no. of plague cases	33	23
Total no. of plague deaths	6	8

*Cases occurring within 1 day to 5 months after cyanogas fumigation.

Table 50. Plague attacks and deaths before and after cyanogas fumigation in four districts of Mysore State, 1948-49

District	No. of localities fumigated	Before cyanogas fumigation		After cyanogas fumigation	
		Plague attacks	Plague deaths	Plague attacks	Plague deaths
Hassan	1	1	0	—	—
Kolar	2	15	8	5	3
Mysore	7	49	11	59	26
Chitradurga	13	21	16	223	102
Total	23	86	35	287	131

amply used in ships for fumigation. In India sulphur containing candles called 'Neem battis' or 'Bhoosa battis' were utilised for fumigating rat burrows but these were found to be markedly less effective than calcium cyanide.

Other Methods

Among other methods 'electrocution', use of sticky substance and flame thrown up have been used but these cannot be used for general control measure.

Rat Proofing

The aim of rat proofing is to separate rats from man. The essential measures for rendering buildings inaccessible to rodents (particularly rats) are following:

a) Provide concrete floors and/or to install L-shaped curtain walls, the lip of which should be at least 60 cm deep and protrude 30 cm outwards (or better 60 cm).

b) Closing all openings and vents leading into the room with cement or cement and broken glass. Wooden sills and doors at ground level should be protected by sheet metals. Openings of sewers must be adequately guarded with solid grids.

c) Use of 'rat guards' on cables in moving ships prevents rat infestation.

d) Blocks of houses or settlements may be protected by surrounding them with trenches 45 cm wide and 1 m deep with vertical sides.

e) Pucca buildings prevent rat harbourage (particularly of *R. rattus*) while thatched roof and other temporary roofs provide shelter to commensal rats.

f) Food storage constructions should be guarded by rat-proof devices.

g) Public health laws must provide clauses for compulsory rat proofing of godowns and storage houses.

Control of Wild Rodents

Systematic campaigns for rat control and rat destruction measures should be adopted by the state control organisation to search for wild rodent foci and to eliminate them by poisoning and fumigation at regular intervals in conjunction with surveys and investigation supported by laboratory and field serological work, till the foci are completely eradicated. Such measures have been undertaken in California (USA), South Africa and Caucasian Steppes and also in the water sheds of Mysore in India.

Administrative Measures, Planning and Coordination

Adequate administrative measures for planning and coordination for rat destruction and rat control are essential for state health department.

Vector Control by Pesticides

The first effective field operation of DDT for the control of plague was made by US Army in occupation of Dakar (West Africa) in 1944. The floors and walls were strewn with 10 per cent DDT dust, leading to the stoppage of plague infection in two weeks. The residual effect in a dilution of 1 : 200,000 in sand was found to last for 230 days by Sharif (1941-43) in laboratory experiments at the Haffkine Institute, Bombay, while the effect of BHC in concentration of 1 : 5000 lasted for 48 days only.

Experimental work to study the pulicicidal effect of DDT in natural rat holes in the field was carried out by Madras workers in 1947, when DDT was used in 5 per cent concentration in kerosene. The results obtained showed that out of 173 rat holes insufflated with DDT at intervals of 2 to 8 weeks live fleas were found in 14 out of 45 rat nests. Based on these preliminary findings rat burrows in 500 houses were insufflated with 10 per cent DDT dusting powder. The flea indices which were 3.1 and 3.6 before insufflation came down to 0.65 and 0.3 respectively as against 3.6 and 3.5 in the control villages.

The overall results show that out of 180 villages which were given treatment under different forms, 27 reported infection after treatment and 153 escaped infection altogether. The above villages before and after treatment with DDT by indoor residual spray alone or by combination on with insufflation of rat burrows with DDT and rat proof treatment showed considerable reduction in flea indices. However, in some villages infection in rats and human beings did not come to an end in majority of cases even with 10 per cent aqueous suspension of DDT.

Similarly the results obtained in Mysore, Punjab, Hyderabad and Bombay state (Wagle and Seal, 1953) indicated a great reduction of flea indices after DDT treatment but the residual effect of the spray remained for 3 months.

Comparative Value of DDT, BHC and Cyanogas as Pulicicidal Agents

A comparative study of DDT insufflation and cyanogas fumigation was made in the Nilgiri district in 1948-49. The results show that DDT was

very affective in keeping general flea index below 1 for nearly 16 months after a single application whereas with cyanogas fumigation the monthly flea index in Coonoor town remained quite high. Again, while plague occurred in 45 out of 460 village units regularly fumigated with cyanogas once every 3 or 4 months all the 90 village units treated with DDT escaped infection during the same period.

On the other hand, to test the value of gammaxene (BHC) as an insecticide for control of plague the field experiment was carried out by Madras workers. The results show that the general flea index was lower in the treated villages than that in control villages but the reduction was not so much as that resulting from DDT treatment. Similar experiment in the Punjab state showed that the residual action of both DDT and BHC was evident till 84-90 days after the treatment, as the flea indices remained practically at the same level as that obtained 3 weeks after treatment. The effect was found lost in the second survey carried out after 184-193 days. It was concluded that both 10 per cent DDT dusting and 10 per cent BHC dust showed evidence of residual action for at least 3 months, the former showing comparatively better results. Both BHC and DDT were better than cyanogas dusting, as also noted in the experiment carried out in Uttar Pradesh (Wagle and Seal, 1953).

Resistance in Flea Population

Since several instances of development of resistance in flea population have recently been detected in India (in Mysore and Bombay States), the proposition should be met by an immediate change and intensive use of insecticide and a close vigilance kept in other areas so that necessary steps may be taken on the slightest suspicion of resistance being developed in the flea population. Application of DDT in smaller doses than necessary or use of adulterated or bad quality product may ultimately prove to be dangerous and uneconomical as it has happened in case of malaria eradication programme. Certain amount of complacency developed on the advocacy of collateral effect of DDT residual spraying in malaria eradication programme on the reduction of flea indices, but if antiflea measures are to be undertaken it should be done on a thorough basis with proper dose of the insecticide and should be sprayed all the year round instead of depending upon two or three rounds of DDT as a part of anti-malaria operation (The anti-malaria operations having been stopped long ago, the so-called advantage of residual effect of DDT spraying in relation to eradication operation will not arise at all in anti-plague surveillance operation, if needed). This is sufficiently indicated in the results of experimental operations described earlier. Besides, the areas involved in sylvatic and wild rodent plague may not have any malaria problem and may be away from human habitation. According to the work of the author and of Baltazard (1960), the reservoir of infection being the rodents, wild as well as commensal, one of the essential steps needed for the eradication of plague is the systematic destruction of these

rodents as was done in Calcutta (West Bengal surveillance operation described in Chapter XIII) and by Fenyak (1960) in USSR, the main target of attack being the vector fleas and destruction of rats.

Control of Plague through Prophylactic Vaccines

The antiplague vaccine prepared by Haffkine in 1897 is one of the earliest antibacterial vaccines for prophylactic use in human beings. This was prepared at a time when the knowledge about the organism, its culture medium, the growth factors and the antigenic structure was not acquired. With the improvement in such knowledge considerable improvement has been effected in the vaccine preparation and in the assessment of its potency. Although studies on statistical evaluation of organised field trials are not many there is sufficient evidence, both experimental and field operational, to show that the new improved quality of Haffkine vaccine in casein hydrolysate medium developed by the author (Seal 1941-42, 1950) is of established value in the prevention of plague. This has been supported by other workers like Dieudonne and Otto (1928); Girard and Robic (1936); Otten (1936, 1941); Patel and Rebello (1948); and Meyer (1953).

The types of vaccines which have been used from time to time are:

1. Killed plague vaccine
   a) Haffkine broth vaccine,
   b) Casein hydrolysate direct vaccine, and
   c) Agar-grown vaccine.
2. Live avirulent plague vaccine (Girard and Robic, 1936; Otten, 1936).
3. Specific protein of plague organism—chemically extracted.
4. Other killed vaccines tried
   a) Pseudotuberculosis vaccine (Boye, 1932; Thal, 1955).
   b) Lipo-vaccine (Boye, 1933; Pons and Advier, 1933).
   c) Sugar vaccine (Minervin *et al* 1935; Korobkova, 1940).
   d) Precipitated vaccine—alcohol and alum precipitated. (Wayson *et al* 1946) and
   e) In oil adjuvant (Spivack and Karler, 1956).

Killed Vaccine

The conditions precedent to the preparation of killed vaccine are selection of a highly virulent plague organism and suitable medium. Though toxicity and potency of vaccine was considered to be interrelated, the more recent work of Sokhey and Habbu in 1950 shows that they are not interrelated, so far as the mouse protection is concerned. A standard method of biological assay of plague vaccine has been worked out by Sokhey (1949).

Toxicity test of different antiplague vaccines revealed that agar-grown vaccine is least toxic and next best is the casein hydrolysate direct broth vaccine incubated at 32°C lately used in India. It is practically free from non-specific protein, less toxic, more potent and less costly. One more advantage of the liquid medium is the elaboration of toxin converted into toxoid by

formalin, which may be helpful in raising the immunity against the possible toxin as well. The author, however, thought that there is still some scope for improvement of both broth and agar-grown vaccines, particularly in respect of better medium for growth. There is also a lot of variation in the quantum of organisms, included per ml of the vaccine. In any case, according to Meyer (1953) none of the vaccines, given in two doses, contains enough specific antigen to produce fully-effective immunity. Experiments carried out in human volunteers show that the quantity of vaccine which contained 2.3 mg of fraction 1 (fraction A of Seal) is capable of favourably altering the susceptibility of 50 per cent of inoculated persons. If this has to be achieved with killed vaccine, reinoculation at intervals of 3-6 months is essential. No adverse negative effect was observed in a mass-vaccination campaign with Haffkine vaccine (Patel and Rebello, 1948), agar grown vaccine (Pollitzer, 1949) or, killed or living vaccines (Meyer, 1953). It was also further confirmed in field observations by Simeons and Chattre in 1946, 1947.

Live Avirulent Vaccine

Girard in Madagascar with avirulent EV strain and Otten in Java with avirulent Tiwidej strain obtained great success with live vaccine. Similar vaccine has also been used in Argentina, Belgian Congo, Brazil, West Africa, Tunisia and Union of South Africa (Pollitzer, 1954). But Sokhey and his co-workers in India did not agree to try such vaccines due to certain amount of risk involved, such as the possibility of changing into virulent form. Besides, greater control and supervision for its preparation and better storing facilities are necessary. Also, without freeze-drying, it is not possible to keep the live vaccine for use in distant parts of this tropical country.

Specific Soluble Protein for Human Immunization

The author (Seal 1943, 1951a, 1953) produced experimental evidence to show that the immunizing substance in the Haffkine plague vaccine is a specific soluble protein and the potency of the vaccine depends entirely on this specific substance. According to Meyer 2-3 mg of this specific antigen is capable of producing a high protective immunity in man. The possibility of its use in human immunization against plague infection needs to be explored. Even the bacteria-free filtrate of casein hydrolysate broth vaccine may be dried and the crude protein may be used in quantities required for producing sufficient immunity. The present status of therapy of plague including the prophylactic vaccines has been thoroughly reviewed by the author (Seal, 1960a).

Control of Pneumonic Plague

Pneumonic plague is one of the easiest epidemic to control if precautionary measures are undertaken at the very outset. The principles are:

1. Compulsory and complete isolation of active and suspicious cases.
2. To attend cases and suspects under coverage of masks and gowns

after being inoculated with anti-plague vaccine.

3. Start intensive treatment with streptomycin and sulpha drugs and with high potency anti-plague serum intravenously (after taking necessary precaution for serum reaction).
4. Anti-rat measures by cyanogassing and other methods.
5. Protect others in the house and neighbourhood by administering anti-plague vaccine and chemoprophylaxis with sulpha drugs.
6. Provide all arrangement for laboratory diagnosis and special camp hospitals.
7. Strict surveillance operations till the epidemic is fully controlled.

Surveillance of Plague

Surveillance is indispensable for areas where natural plague foci exist or where there is history of past infection. Modern trend in public health emphasises the utilisation of epidemiological principles in organising control and preventive measures through surveys and integration and coordination of all aspects of communicable diseases including plague. It is essential that specially trained personnel be available within the public health services of each country concerned, to carry out emergency control of plague episodes. While many natural plague foci in Western and temperate climate have been fairly adequately studied, the same cannot be said about tropical countries including India. The Bombay city had long been carrying out surveillance work on a small scale by trapping and examining the rat population regularly for the presence of plague infection by dissection and culture. However, the author following the reappearance of plague in Calcutta in 1948 established a Plague Control Laboratory under the Govt. of West Bengal in 1949 to carry out systematic investigation and thorough surveillance operation with a view to eradicate the infection from among the rat population.

These studies provide sound basis for plague control programme and indicate the need for long term ecological studies in crucial areas with a view to finally eradicate the disease. In fact, the surveillance operation undertaken by the author and supported by experimental work on rat movement, and ecological studies on rats and fleas now provide the clue for ultimate eradication of the disease (Seal, 1980).

The results of surveillance programme in Calcutta and surrounding areas during the period 1950-78 are briefly discribed in the following chapter.

CHAPTER XIII

Surveillance Operations

The control and surveillance work during the post-epidemic phase was started in 1953 and is still continuing on a limited scale (Seal, 1980) on the following lines.

1. Regular trapping of rats and collection of dead rats, if any, from streets and houses of different wards and areas of the cities of Calcutta and Howrah and also from the neighbouring municipalities throughout the year by field workers employed by the Plague Control Laboratory for dissection, bacteriological examination, species identification, and sex differentiation *etc.*

2. Examination of the fleas, identification of species, sex differentiation and isolation of plague bacilli by microscopic and culture examination and also for calculation of specific and general flea indices by season.

3. Examination of splenic tissue, smears and blood by microscopy and cell culture technique for isolation of plague bacilli.

4. Carrying out the virulence test of the organisms isolated from rats, fleas and human cases.

5. Insufflation of rat burrows and disinfestation of premises of the affected wards and areas with DDT dust.

6. Regular and repeated cyanogassing of rat burrows.

7. Detection of human cases, if any, their diagnosis and treatment and associated prevention and control measures.

8. Immunisation of persons living in the affected areas with Haffkine Institute plague vaccine.

9. Carrying out ecological investigations and other researches on rodents, fleas and improvement in the bacteriological technique.

10. Carrying out health education measures particularly for eliciting cooperation of the people of the affected area in handling dead rats and other methods of prevention.

Additional measures

Between 1970 and 1976 the following additional measures were also undertaken to assess the state of plague endemicity in the two cities following the control and surveillance operations for a period of about 20 years:

(i) testing of local rodents for susceptibility or resistance against plague infection by the standard test advocated by the Haffkine Institute, Bombay;

(ii) testing for the presence of antibodies against plague bacilli on a mass scale by the newly developed sero-agglutination and gel-precipitation technique developed by Seal (1951a; Seal, 1972a) and by other serological test for comparison.

Collection, Identification and Destruction of Rats and Fleas

The total number of rats collected and destroyed in 23 yr was 29,23,753 —an average of 1,27,120 per year. Excluding the lean years or work namely, 1950, 1970 and 1971 the average number of rats destroyed per year was 1,36,753. The number of live rats collected was 8,25,412 giving a proportion of 28.2 per cent of all rats (living, dead or killed). During this period the total number of fleas examined and identified was 3,82,843 and the flea indices varied between 4.92 in 1948 to a minimum of 0.1 per rat in 1970. The total number of rats examined for fleas was 7,73,879 giving a general flea index of 0.05 on an average.

Changing Pattern of Rodent Species in Calcutta and Howrah between 1949 and 1971

The comparison of distribution of rodent species in Calcutta and Howrah during 1949–1971 with that during 1906–1936 showd that the proportional distribution of *R. rattus* was almost same in 1949 as in 1906–1936. It dropped from 7.3 per cent in 1950 to 2.2 per cent in 1964 and 0.8 per cent in 1971. The distribution of *R. norvegicus*, on the other hand, had already come down from 26.0 per cent in 1906 to 22.0 per cent in 1936 and it continuously dropped and reached the level of 2.82 per cent in 1971. In fact, replacement of *R. rattus* and *R. norvegicus* with *B. bengalensis* led to the compensatory rise in the latter species from 27.3 per cent in 1936 to 97.4 per cent in 1971. It is a significant change making the persistence of plague in the area a remote possibility.

B. indica which was reported by Rao in 1936 to be 37.2 per cent, virtually disappeared leaving only 0.3 per cent in Calcutta and nil in Howrah, in 1971. The changes in the Howrah city ran parallel with that of Calcutta except that *R. rattus* registered a little rise to 1.04 per cent in 1971 against 0.2 per cent in 1965 and 0.25 per cent in 1969. The housing system in Howrah comprising largely of huts in bustees provided facilities for the harbourage of house rats (*R. rattus*). A quick change in housing conditions being not possible continuous surveillance operation was needed for rat destruction.

Rodent Densities in Calcutta and Howrah

The comparison of rodent densities of rats in Calcutta and Howrah during 1960–71 with that during epidemic years (1948–50), showed that the overall density during 1948–50 when epizootic had been going on, was somewhat lower than that during 1960–66. It may partly be due to the effect of the

epizootic. From the average annual densities as recorded between 1960–71, there is an indication of an overall reduction in densities in the latter part of the decade than in the earlier part. It is also seen that the density of rats in Howrah was consistently higher than that in Calcutta. This was probably because not only huts and *bustees* but also large godowns, mills, factories and railway goods sheds within Howrah city harboured large number of rats. It was found that 1964 was the year of peak density for both Calcutta and Howrah, the respective figures being 144 and 169 per 100 traps. The high density was, however, followed by a fall even lower than that of the previous years indicating a possible periodic accummulation followed by a natural destruction to maintain a rational balance between space (shelter), food and number.

Seasonal Variation in Density of Rats

The sex-wise annual distribution of rats between 1965 and 1971 when analysed showed that the proportion of females was always larger than that of the males. The average for 7 years between 1965 and 1971 was 1.47 females against 1 male; it was of course higher, *i.e.*, 1 : 1.61 for the epidemic years. Taking Calcutta and Howrah separately the number of females was proportionately higher in Calcutta as compared to Howrah (average 1:1.51 against 1 : 1.33). A difference was, however, noted in the M:F ratio in different months of the epidemic year (Table 51).

The Table shows that during the epidemic years M : F ratio of the rodent population as a whole increased progressively between January and April from 1 : 1.3 to 1 : 3.13 and declined to 1 : 0.80 in August followed by a rise

Table 51. Seasonal distribution of female rats against male rats in 1948-50 (plague epidemic years) in Calcutta and Howrah

Months	Rr	Rn	Bb	Bi	All species
January	1.19	1.53	1.27	1.28	1.30
February	1.73	1.78	2.10	2.00	2.06
March	3.00	4.00	2.20	2.75	2.54
April	3.70	1.32	3.00	2.00	3.13
May	2.38	3.17	2.20	1.33	2.33
June	1.10	1.78	4.78	4.50	1.66
July	1.66	2.07	1.00	1.27	1.15
August	0.72	1.16	0.75	2.00	0.80
September	1.00	1.50	0.90	1.36	1.00
October	1.28	0.98	1.16	1.30	1.23
November	1.16	1.20	1.22	1.31	1.23
December	1.3	1.54	1.28	0.72	1.20
Average of species	1.91	2.08	1.47	1.56	1.56
Number examined	1286	982	6457	235	8960

in the last quarter of the year to 1 : 1.23. As far as the species was concerned the same trend of seasonal variation was maintained by *R. rattus* and *B. bengalensis*. *R. norvegicus* also showed similar trend except that the peak of female preponderance 1 : 4.0 was seen in the month of March and the minimum (1 : 0.98) in the month of October. *Bandicoota indica* showed an irregular trend, the maximum M : F ratio of 1 : 4.5 being attained in the month of June and the minimum 1 : 072 in the month of December. The average for different species ranged between 1 : 1.47 for *B. bengalensis* to 1 : 2.08 for *R. norvegicus* closely followed by 1 : 1.91 for *R. rattus*.

Control Measures

Disinfestation of Premises and Insufflation and Cyanogassing of Rat Burrows and Immunisation of Population

During 1949-1971, a number of premises were disinfected, rat burrows insufflated with DDT or cyanogassed and persons immunised against plague in Calcutta and Howrah. In spite of long absence of human cases and discovery of infection among rats and fleas, DDT dusting and cyanogassing operations were continued unabated. The number of persons inoculated against plague was, however, greatly reduced, this being done only in the locality or around the houses where dead rats were detected. The average number of premises disinfested, rat burrows insufflated and rat burrows cyanogassed per month was 579, 1561 and 14736 respectively. It may also be mentioned here that in addition to the above measures poison baiting was also undertaken on a small scale in some areas where rats caused household damages.

Assessment of Resistance in Local Rat Populations against Plague in Calcutta and Howrah

In all phases of epidemiological studies on plague namely, pre, intra, post and inter-epidemic, the estimation of herd immunity among the local rodent population and of the prevalence of plague infection assists in the prediction of the future trend of plague in the area. Thus timely measures can be planned and adopted. The methods adopted for the assessment of herd immunity along with their comparative efficiencies, advantages and disadvantages and the results obtained are presented below. The six methods which were used are: (i) Biological method of the Haffkine Institute; (ii) sero-agglutination test; (iii) sero-precipitation test; (iv) complement-fixation test, (v) haemagglutination test; (vi) gel-precipitation test; and (vii) Ring-precipitation test.

(i) *Biological method:* The protective value or the specific antibody content of the sera of rats was tested in in-bred white mice by the method standardised by Sokhey (1939a) and Habbu (1960) and LD_{50} values were estimated by Reed and Muench's method. The test required a period of about 30 days for drawing up the final result. To assess the resistance or susceptibility

of rats the method used by Habbu (1960) is generally used in India.

(ii) *Sero-agglutination test:* After the main difficulty of agglutination reaction was removed by introducing new culture media by the author (Seal and Mukherji, 1950) a quantitative agglutination test described earlier was developed to estimate the antibody content of sera, collected from the rats in the field.

(iii) *Sero-precipitation method:* This was successfully developed by the author (Seal, 1951a) by using specific plague protein antigen I or antigen A diluted to contain 0.1 mg/ml (1 : 10,000) for quantitative precipitin N estimation giving the same result as the biological test in a much shorter time (3 days). For quicker result ring precipitation test could also be used.

(iv) *Complement-fixation test:* Various workers tried to utilize this test for estimation of the antibody content of sera are Greval and Dalal (1933), Joltrain (1936) and Chen *et al* (1952). The author (Seal, 1953) with his newly isolated specific protein confirmed the usefulness of this test by using antigen A or I for sensitizing the red cells.

(v) *Haemagglutination test:* Originally used by Keogh *et al* (1947, 1948), the sensitization of red cells by antigen I gives the antibody titre of the serum on a rough estimation.

(vi) *Gel-precipitation test:* A new gel-precipitation test for detection of immunity status of rodents against plague infection was discovered by the author (Seal, 1972a). The method is briefly described below:

(a) Preparation of antigen—*Y. pestis* grown on casein hydrolysate agar (Seal, 1950) at 37°C for 48 to 72 h is washed in 20 ml of distilled water and after purifying it is suspended in double-distilled water at a concentration of 500-600 million organisms per ml, and left in the incubator at 37°C with occasional shaking till free from live organisms which usually takes about 12 days. The emulsion is then filtered through Seitz filter and precipitated with 33 per cent Na_2SO_4. This precipitate recovered over a filter paper is suspended in distilled water and dialysed in cold till sulphate free and then dried in a vacuum desiccator over P_2O_5, and finally weighed.

(b) Preparation of the gel-plate—A gel prepared with 1 per cent bacto-agar and 0.1 mg/ml of the above specific protein, steamed at boiling temperature of water is mixed with 100 units of penicillin-cum-streptomycin per ml after cooling and poured into petri-dish as a thin layer and allowed to solidify. About 16 to 20 holes are cut into this gel as in gel-flocculation test.

(c) The test—Sera collected from rats and preserved in the refrigerator are separately placed into the holes of the plate by using separate capillary pipettes for each of the dilutions of 1 : 20 and 1 : 40. In the central hole a known antiplague serum is placed as control. Similarly other known control sera may also be used. The petri-dish is then placed in the incubator for 24 to 48 h. The positive result is indicated by a white precipitation ring around the hole, the depth of which roughly gives an idea of the intensity of precipitation reaction and of the level of immunity (Fig. 43).

(vii) *Ring-precipitation test:* For quicker diagnosis ring-precipitation test

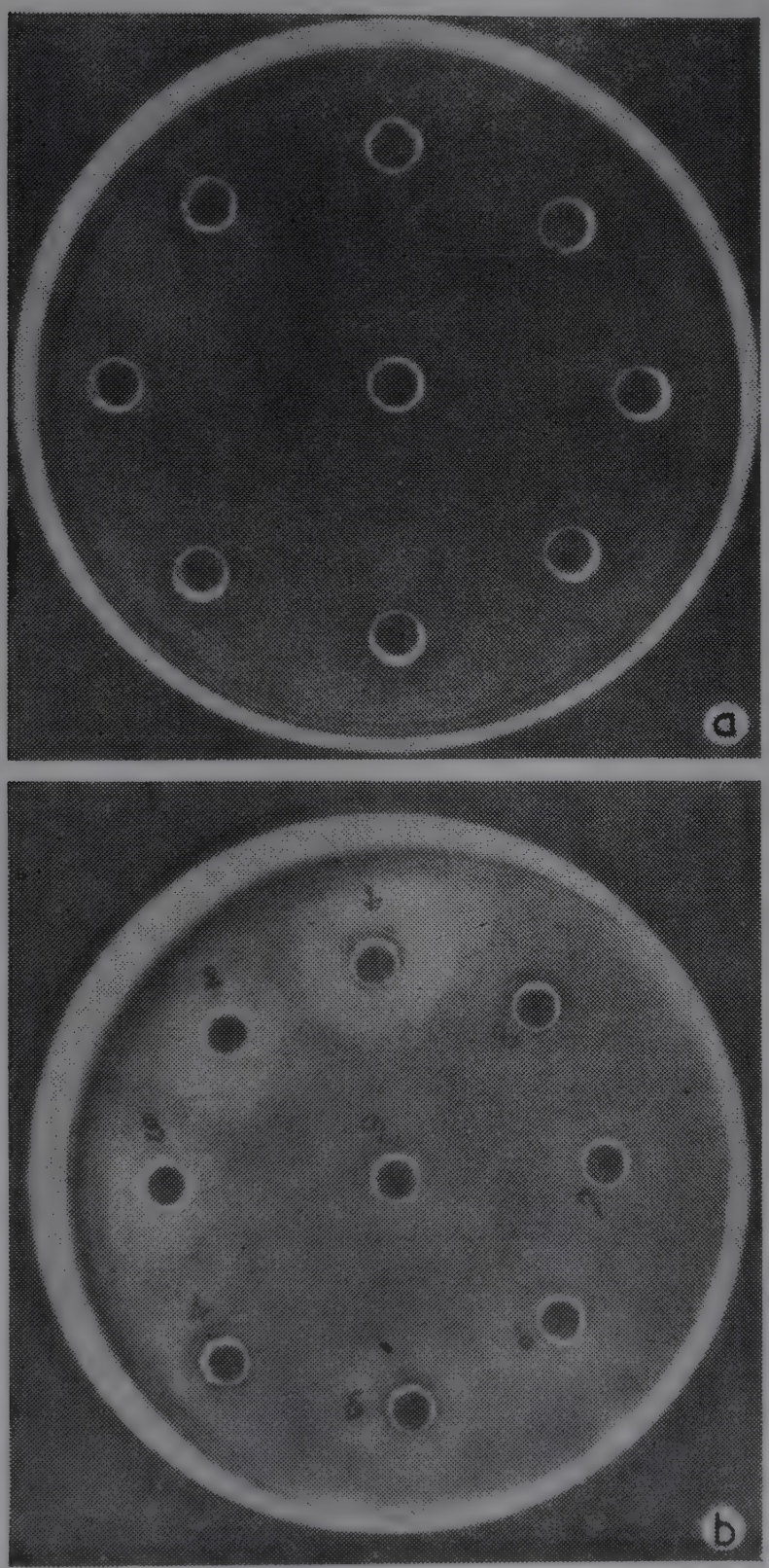

Fig. 43. Gel-precipitation test: (a) wells out before testing; (b) gel-precipitation (positive and negative).

can also be done by using the above antigen at a dilution of 1 : 1000 in Dreyer's tubes in 0.25 ml amount. The unknown serum in dilution of 1 : 19 and above is added by means of a capillary tube and readings taken after ½ an hour at room temperature.

Comparative Values of the Results

All these tests were independently utilised for testing groups of rat sera collected from the fields at Calcutta and Murarai (Birbhum). The comparative values obtained by different tests are given in Table 52.

The Table shows that the percentage of resistant rats detected by biological method was about 21 whereas it ranged between 29.6 and 34.0 by sero-agglutination, complement-fixation and gel-precipitation tests. It may, however, be mentioned that the sera collected from Calcutta were old and preserved for a long time before use, whereas those from Murarai were fresh, but the two results corroborated with one another. However, use of 5,000 live virulent *Y. pestis* appears to be too stringent for rats. For haemagglutination test 25 sera—15 serologically negative and 19 serologically positive, were sent to the National Institute of Communicable Diseases (NICD), Delhi, where this test was being routinely done. The results corroborated in respect of all the negative sera and discrepancies were noted in respect of one or two of the positive sera. Again, the sera were too old when it was decided to send them to the NICD at Delhi.

The advantage of the gel-precipitation test is that once the antigen is available the test can be done even in the field and several hundred of rats can be tested in 2 or 3 days. It is positively useful for surveillance operation in plague control work anywhere in the world.

Table 52. Comparative values of the results of immunity tests in rats against plague infection in Calcutta and Murarai in 1971

Methods used	Per cent rats found resistant		
	B. bengalensis	*R. rattus*	Total
Calcutta sera:			
Biological test	19.3	37.5	20.9
Sero-agglutination test	32.7	42.1	34.0
Complement-fixation test	28.3	36.4	29.6
Gel-precipitation test	30.0	42.1	31.9
Murarai (Birbhum) sera:			
Biological test	19.5	24.8	22.0
Complement-fixation test	23.3	25.0	23.0
Gel-precipitation test	23.5	27.3	24.4

CHAPTER XIV

Current Position of Plague in the World

In spite of the comprehensive knowledge now available about plague as a disease namely, history, etiology, source, transmission, geographical distribution and other epidemiological characteristics, treatment, control and prevention and finally effective surveillance operations that may lead to its eradication, there are several countries in the world where the disease is still continuing to occur causing undesirable loss of human lives and dislocation of human activities. The fatality rate has, however, largely reduced due to the effective treatment and preventive measures, but the bottleneck remains with the failure to remove the key factor, that is, the rodent-man transmission.

During the last two decades human cases of plague have been reported from at least four continents—Africa, North and South America and Asia. The countries from where cases have been reported occasionally at regular intervals as published in the Epidemiological and Statistical Reports of the World Health Organization between 1960 and 1982 are listed below:

Africa —Angola, Congo, Kenya, Lesotho, Madagascar, Malawi, Mozambique, Rhodesia (Zambia), South Africa, Southern West Africa (Namibia), Sudan, Tanzania and Zaire.

N.America (*USA*)—Arizona, California, Colorado, Nevada, New Mexico and Oregon.

S.America—Argentina, Bolivia, Brazil, Colombia, Ecuador, Peru and Venezuela.

Asia —Burma, India (till 1968), Indonesia (till 1961), Iran (1965 only), Kurdistan (1963 only) and Nepal (1967 only).

The annual distribution of cases in the four continents during the years 1960 to 1982 is given in Fig 44. and their quinquennial distribution in Table 53 and Fig. 45. No human cases have, however, been reported from Sri Lanka, Australasia, Philippines, Japan, China, Canada, Mongolia, USSR or any other European country during this period. World distribution of plague during 1980–82 is shown in Fig. 46.

It is apparent that plague is persisting mostly in three continents *viz.*, South-East Asia, South America and Africa is that order. Although it is on

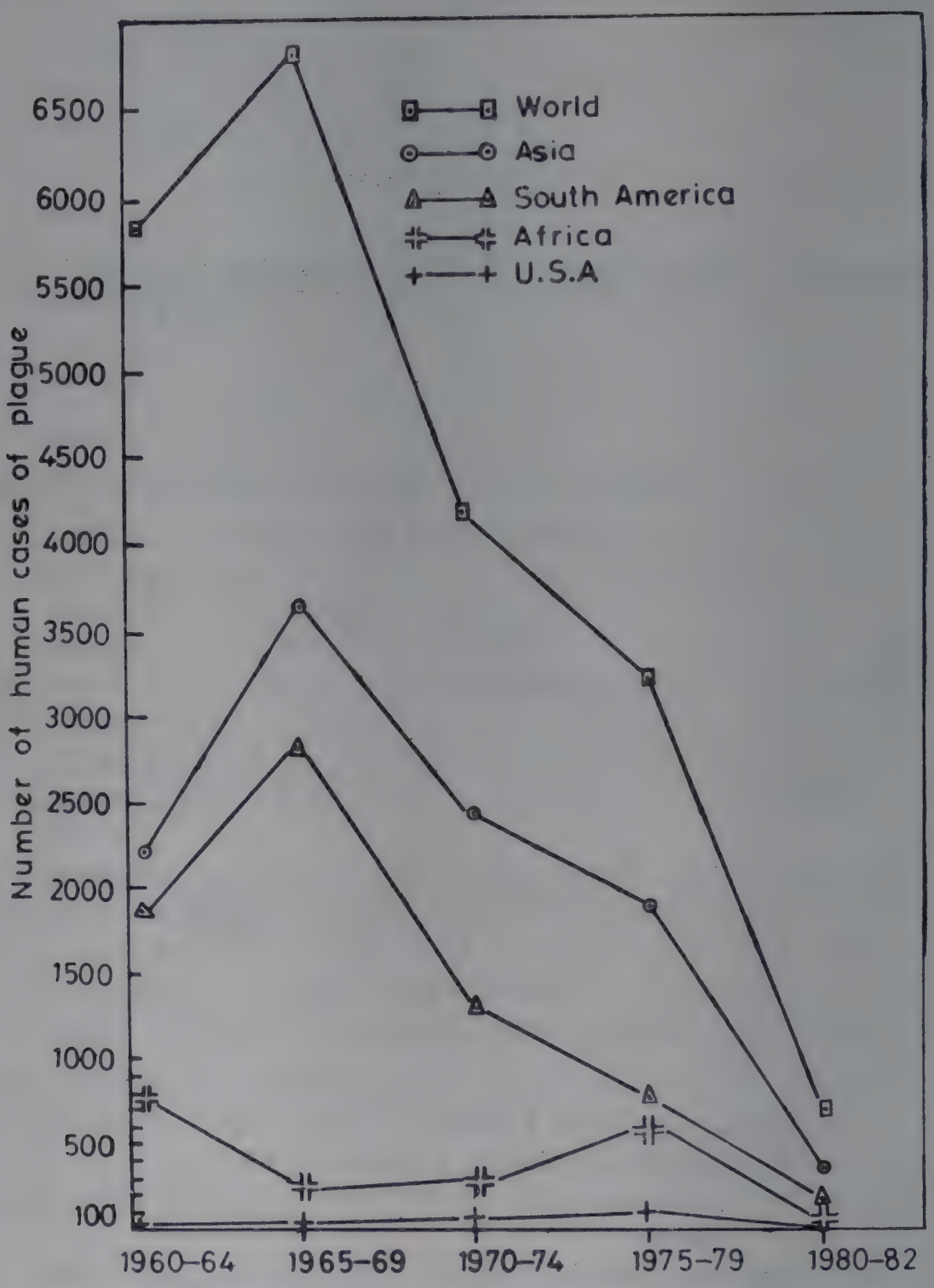

Fig. 44. Quinquennial distribution of human cases of plague in the world in the 4 continents of Asia, South America, Africa and USA, during the period 1960 to 1982.

the decline in most of the countries the worst affected states are Vietnam followed by Burma and South American States of Brazil, Bolivia, Ecuador and Peru in that order. In the United States of America there have been only stray cases in six Western and South-Western States, namely, Nevada, Oregon, Arizona, California, Colorado and New Mexico, the source of infection being generally wild rodents.

Table 53. Quinquennial distribution of human cases of plague in various continents during 1960-1982
(*vide: WHO Epidemiology and Statistics Reports*)

State	Quinquennial period				
	1960-64	1965-69	1970-74	1975-79	1980-82*
Africa:					
Angola	—	—	—	49	—
Congo	29	118	—	—	—
Kenya	31	1	—	64	3
Lesotho	—	7	11	8	—
Madagascar	53	103	112	147	63
Malawi	30	—	—	—	—
Mozambique	—	—	—	112	—
Namibia (South-West Africa)	106	—	23	4	—
South Africa	11	—	—	—	—
Sudan	—	—	—	149	—
Tanzania	515	7	32	2	39
Zaire	2	—	74	18	2
Upper Volta	—	1	—	—	—
USA (North America);	6	24	26	65	8
South America:					
Argentina	12	—	—	—	—
Bolivia	134	280	85	116	45
Brazil	482	902	859	593	120
Colombia	8	—	—	—	—
Ecuador	796	606	67	8	8
Peru	377	956	306	5	31
Venezuela	6	—	—	—	—
Asia:					
Burma	265	317	986	1696	104
India	1460	25	—	—	—
Indonesia	8	42	11	—	—
Vietnam	463	3189	1473	229	254
Others (Iran+Kurdistan)	52	12	7	—	—
Total	4846	6510	4072	3265	677

*Available for 3 yr only.

While most of the states in Africa show a positive trend of decline and are free from human cases at intervals, Madagascar and Tanzania have never been free from human cases and a few other states have been the target of fresh outbreak *e.g.*, Malawi in 1963 and Sudan in 1979. Also the state of Zaire which was free from plague in the earlier decade became involved in 1970 and cases were reported till 1982. In other words, unless a coordinated, conjoined and simultaneous surveillance operation is undertaken as in case of smallpox or malaria there will always be possibility of resurgence of old foci in any state or transfer of infection from one state to another thus

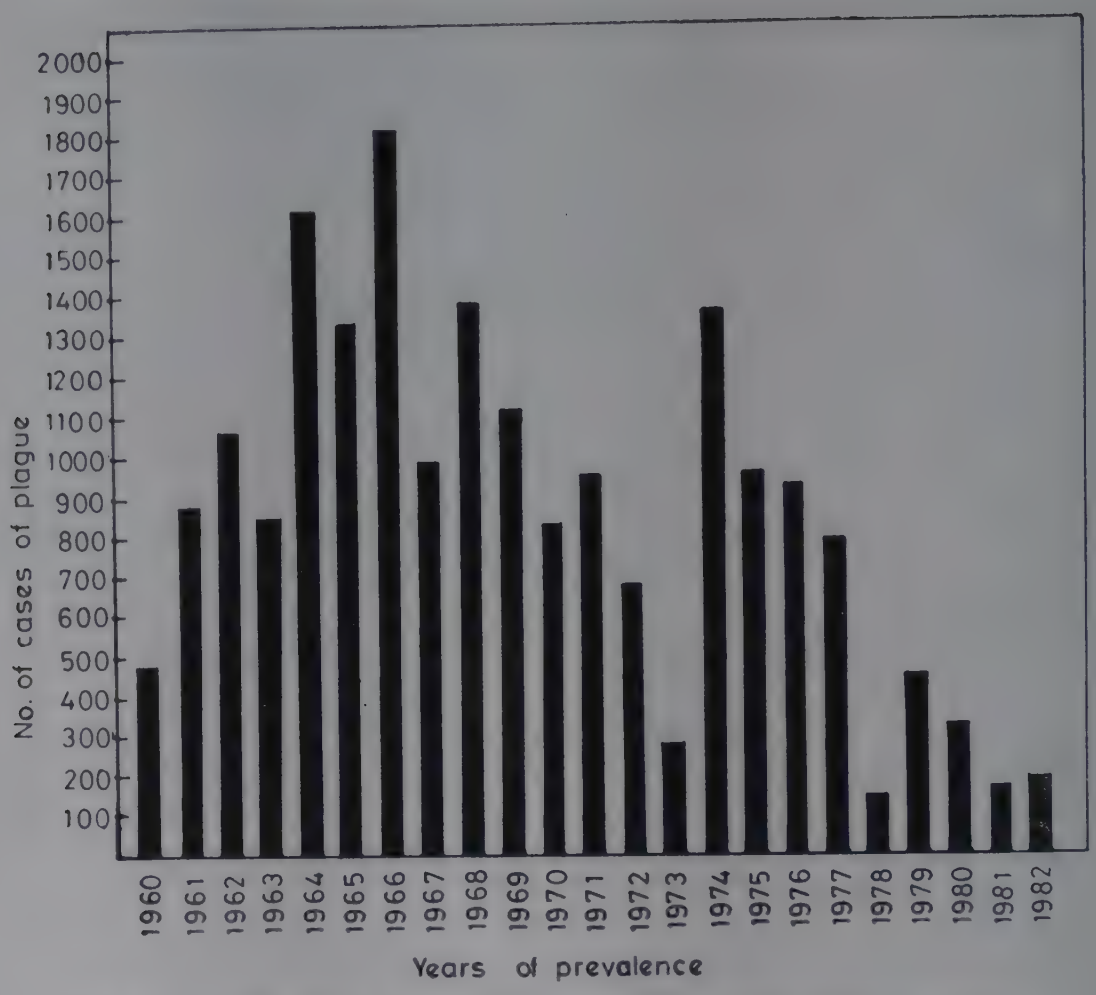

Fig. 45. Annual world distribution of reported human cases of plague between 1960 and 1982.

perpetuating the infection and endemicity. In this respect India which has remained free since 1969 apprehends the danger of importation of infection from the neighouring states of Burma and Vietnam where plague is prevailing unabated. However, considering all points plague is currently at comparatively low ebb as shown in Fig. 46 and presumably it might be considered as the opportune moment for an all out action by the World Health Organization if complete eradication of plague from the world is desired.

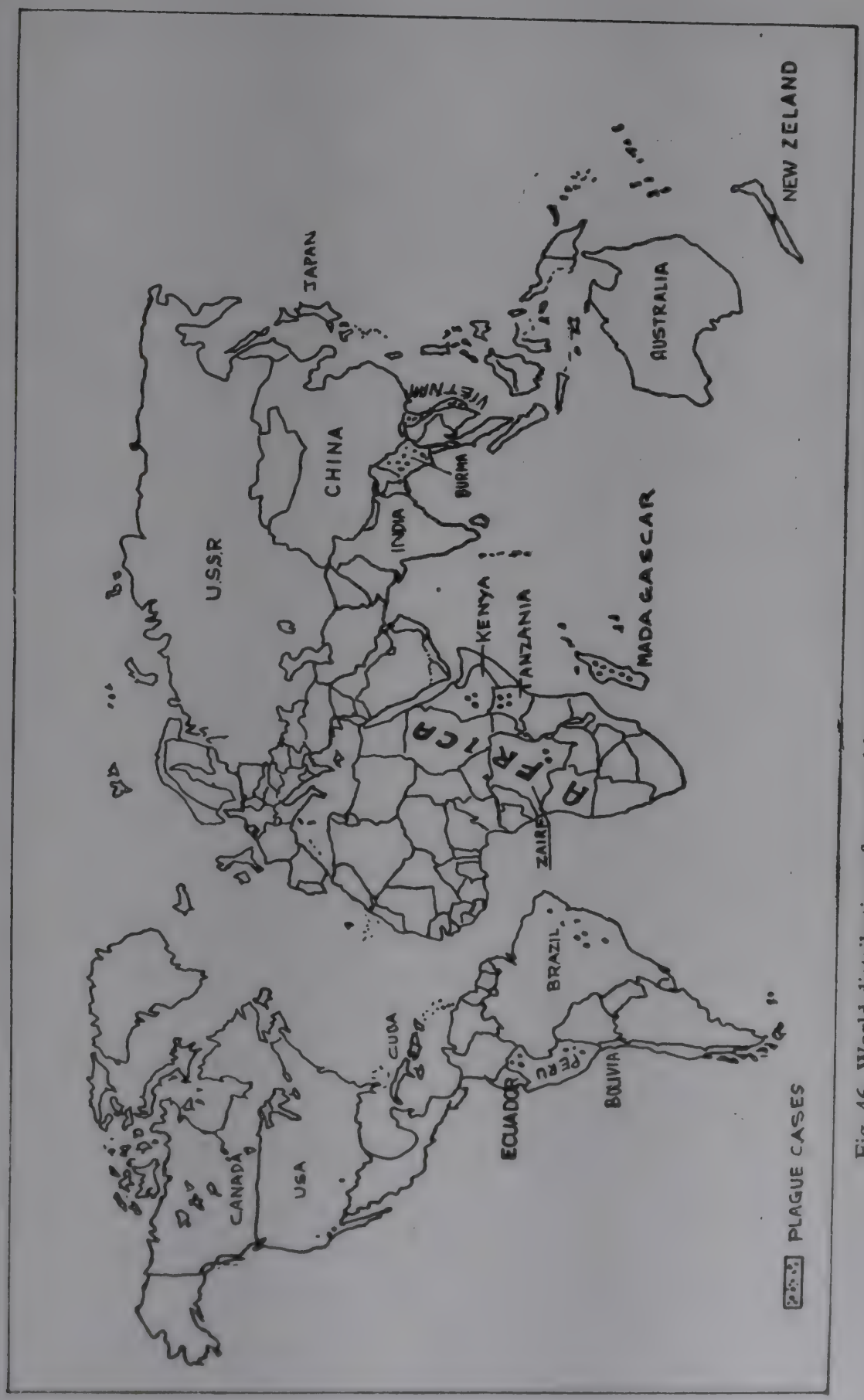

Fig. 46. World distribution of reported human cases of plague during 1980 to 1982.

Epilogue

Plague has been with us as long as history. In the middle ages it devastated Europe and of the three main pandemics the last one which started towards the end of the nineteenth century covered the widest area of the world and India particularly suffered most till 1918. Now it will be gathered from the accounts given in this Monograph that the incidence of plague has markedly diminished within the recent years and in some countries specially India, the disease has ceased to manifest for some time. But the latter stands in danger of importation of infection any time through land or sea route from the adjacent countries of Burma and Vietnam.

In Epidemiological studies have revealed decline in the incidence of plague when no anti-plague measures were taken. However, the disease flared up again in due course as it is happening now in many of the African States. In the words of Pollitzer (1954), the veteran scientist, 'while appreciating the strenuous efforts now often made to combat the scourge one should be careful not to ascribe the freedom from plague in any country to the measures taken but as an outcome of the periodicity of infection'.

It must, however, be admitted that since the above statement was made many new observations have been made and newer knowledge gained which actually form the basis of this Monograph. There is every hope that by the meticulous application of the principles and methods described under Chapter on 'Surveillance Operations' along with the modern methods of treatment and prevention, it should be possible to finally eradicate the disease if the financial involvement in such a project could be assured and the concerted action is taken by the health authorities of the countries concerned.

It is also to be admitted that the success will depend on the universal action under the aegis of the organisations like WHO particularly directed towards the destruction of the rodent reservoirs and vector fleas combined with therapeutic and prophylactic measures among the population suffering or under risk. In addition to the campaign against the disease as described above, it would be of further advantage if simultaneous improvement could be brought about in the general conditions of living, particularly of housing which keeps away the rodents, and in health knowledge and practice all of which played major role in freeing Europe from this scourge at a time when the knowledge about its etiology, source, transmission, treatment and prevention was only in the nebulous state.

* AS LONG AS THERE IS ACTION THERE IS HOPE *

—The Author

APPENDIX I

Chronological Order of Development of Knowledge about Plague

1894	—Discovery of *Pasteurella pestis* (now *Yersinia pestis*) by Yersin and Kitasato from the blood, bubo and spleen of human patients.
1895	—Antiplague serum by Yersin, Calmette and Borrel.
1896	—Antiplague vaccine by Haffkine in Bombay.
1897	—Ogata (Formosa) } Fleas as vector of transmission from rat
1898	—Simond (India) } to rat and man.
1902 to 1903	—Gauthier and Raybaund proved transmission by fleas experimentally.
1904	—Durk of Austrian Plague Commission worked out the pathological anatomy of plague.
1905	—Glen Liston (Bombay) and the Indian Plague Commission confirmed rat-flea-rat transmission.
1910	—Rowland postulated endotoxin of plague bacillus.
1914	—Bacot and Martin of the Indian Plague Commission described the role of "blocked" fleas in the transmission of plague infection.
1928	—George (of Africa) described plague in the wild rodents (sylvatic plague).
1930	—Naidu *et al* utilised horses in Bombay for the production of antiserum, the only available remedy till that time.
1932	—Schutze differentiated envelope and somatic antigen.
1935	—Otten in Java introduced live attenuated plague vaccine followed by Girard and Robic of Madagascar and Pirie and Grasset of South Africa.
1932-1935	—Sokhey at Bombay described mouse-protection test, for the standardisation of antiplague sera.
1939	—Sokhey standardised the virulence test and culture medium and described optimum conditions for growth of plague bacillus.
1939	—Schutze (Great Britain) and Sokhey (India) introduced treatment of plague with sulpha drug.
1940	—Bhatnagar (India) classified the plague and pseudotuberculosis organisms by improvised agglutination test.

1940-1941 —Seal and Mukherji in Bombay utilised modified casein hydrolysate medium for the growth of plague strains and the study of chemical antigenic structure. Seal also solved the problem of auto-agglutination of plauge strains by using live organisms against the various types of antisera raised, and developed serological test for identification of virulent plague from the non-protective plague and pseudotuberculosis strain.

1943 —Seal isolated specific soluble protein fraction of plague bacillus and also the fraction correlated with *P. pseudotuberculosis.*

1944 —Jawetz Meyer (USA) differentiated virulence from toxicity of plague bacillus and studied the mechanism of immunity in plague.

1946-1947 —Vishwanath (Bombay) used DDT as an effective insecticide in India.

1947 —Baker *et al* (USA) purified specific protein antigen of plague bacillus.

1947-1948 —Streptomycin was used very successfully in both bubonic and pneumonic plague in India and other places.

1949 —Devignat of Belgian Congo classified plague strains into Orientalis, Antiqua and Mediaevalis on the basis of reaction with glycerine and production of nitrous acid.

1950 —Sokhey *et al* (Bombay) adopted casein hydrolysate medium for the preparation of anti-plague vaccine.

1956 —Burrows and Bacon described two additional antigens Vi, V and W in plague bacillus.

1952-1957 —Seal proved on the basis of experimental epidemiology that partially immune rodents and not fleas act as reservoir of infection during inter-epidemic period by harbouring plague organisms of reduced virulence in an inapparent form or in chronic foci in spleen under the Indian environmental conditions.

1959 —Seal presented evidence of both capsule and envelope being present in virulent plague bacillus.

1960 —Seal made a comprehensive review of the status of 'Plague in India'.

Chronological History of Epidemics of Plague in India

1500-600 B.C.—Recorded in *Bhagvata Purana.*

1031-32 A.D. —Plague reached India from Central Asia following invasion of Sultan Mohammad (Arabian Chronicles).

1325 A.D. —Plague in Malabar following invasion of Mohammad Tughlaq and again after Timur.

1403 A.D. —Sultan Ahmed's army was destroyed by plague epidemic in Malwa.

1617 A.D. —Plague was reported during the Moghul emperor Jahangir's reign from Punjab, Ahmedabad, Surat and Deccan and some other parts of India—described by Edward Ferry, Ambassador to the Moghul Court.

1707 A.D. —Plague in Berhampur.

1812-21 A.D. —In Kathiawar, Gujarat and Cutch—supposed to have been imported from Persia.

1836-38 A.D. —In Mewar and Rajputana—which is known as *Pali Plague.*

1895 A.D. —In Calcutta— diagnosed bacteriologically on the 17th April, 1898, by Dr Neild Cook, imported from Hong Kong.

1896 A.D. —In Bombay, first diagnosed on the 13th October, 1897. From here plague spread rapidly to most parts of India.

1907 A.D. —Peak year of plague in India with 1,315,892 deaths.

1926-27 A.D. —Severe epidemic in Hyderabad and Deccan.

1947-52 A.D. —A temporary rise in incidence of plague in Calcutta and rise in several old foci in India.

1954-58 A.D. —Plague reappeared in Andhra Pradesh and Mysore and imported first time in Gowahati (Assam).

1960-68 A.D. —Sporadic outbreaks in Mysore, Madras, Himachal Pradesh and Rajasthan.

APPENDIX II

The Findings in Calcutta Epidemic (1948-52)

Clinical Diagnosis

a) Out of 812 admissions in hospitals and cases detected in the field during the Calcutta outbreak of 1948–52 information could be collected for 618 cases (1948–140; 1949–417; 1950–61; Fig. 1). The figure shows that at the beginning of the epidemic (1948) milder cases appeared more (41.4%) than in 1949 and subsequent years. The number was reduced in later years with increase in cases of moderate and higher severity. In total, 21.7 per cent were mild, 23.8 per cent moderate and 54.5 per cent of high severity.

The fatality rates among mild, moderate and severe cases were 1.5, 0.1 and 23.7 per cent respectively, the combined fatality rate being 14.7 per cent. The fatality rate before the discovery of the specific drugs like streptomycin and sulpha drugs was nearly 90 per cent. The present overall reduced fatality rate of 14.7 per cent was due to the use of specific drugs. The chance of recovery in mild cases was almost cent per cent with proper use of these drugs.

Types of Plague Cases

Bubonic-91.5 per cent; septicaemic-6.2 per cent and pneumonic-2.3 per cent.

The Main Symptoms and their Distribution

High fever-96.1 per cent; pain-36.5 per cent; headache-11.0 per cent; delirium-5.6 per cent; Mania-6.6 per cent; Apathy-0.5 per cent and others 0.3 per cent.

Involvement of Glands

Glands were enlarged in 99.1 per cent cases, distributed as follows—inguinal (including femoral)-72.0 per cent; cervical-15.4 per cent; axillary -17.8 per cent; other glands including vesicles-4.6 per cent; and multiple glands-10.7 per cent.

Affection of glands depended upon the manner of wearing clothes and place of sleeping and resting. The ratio of males to females affected by plague was 5:1. One of the reasons for lesser number of cases among females was perhaps their practice of more thoroughly covering their bodies, thus

preventing contact with fleas and partly due to the lower M:F ratio of city population (2:1). The main signs of disease were dry furred tongue (64.0%), anxious look (25.1%), injected conjunctiva (47.7%), staggering gait (24.4%), hot dry skin (29.1%), blurred speech (23.3%), scanty urine (7.0%), lung symptoms (7.0%), tremors (3.5%) and others (3.5%).

Vesicles

Two cases of vesicles were detected. In one case the vesicles was on left hand below left cubital fossa and in another on left lateral side of the abdomen. In both the cases it was accompanied by bubo in the left axillary fossa.

Non-Plague Cases

Of the 127 cases diagnosed as non-plague, the clinical severity of 123 cases was analysed as mild (65.0%), moderate (30.0%) and severe (5.0%).

The provisional diagnosis in these cases was as given on page 248.

From the description of clinical symptoms and signs it is apparent that diagnosis has to be based on sudden high fever with painful enlargement of glands (bubo) and signs like dry furred tongue, anxious look, injected conjunctiva, staggering gait and certain mental symptoms. Since bubonic type was in overwhelming majority, bubo with fever, pain and facial syndrome was the mainstay of preliminary suspicion of plague, which can then be confirmed bacteriologically by examining the smear from the puncture material or by culturing them and, if necessary, by mouse or guineapig inoculation test. If both vesicle and bubo are present as shown in the picture earlier it is easy to detect the case. But, as will be seen from symptoms and signs of non-plague cases (*i.e.*, those proved to be non-plague) there were many whose signs and symptoms with enlargement of glands could easily be mistaken for plague, particularly at a time when an epidemic of plague is raging. The procedure to follow, therefore, was to hospitalise such cases on suspicion of plague and treat them until proved otherwise.

Laboratory Confirmation of Plague Cases

As indicated earlier 472 human cases were examined for bacteriological confirmation by gland puncture and blood examination. Out of these *Y. pestis* could be isolated (60.8%) from gland puncture in 271 cases and in 16 others by gland puncture and blood culture. Of these 80 strains of plague bacilli, 29 from humans and 18 from rats were studied in respect of colony characteristics, degree of virulence, and serology and the results compared with the clinical types noted in related patients. The following series of sugars were tested for typical fermentation reaction—maltose, trehalose, dextrin for positive reaction and rhamnose, saccharose, inulin, sorbitol and isodulcitoi for negative reaction. In addition, xylose was also included to distinguish between xylose positive and xylose negative strains if it could be utilised for any distinguishing characteristics of plague strains. The serological test was done with live suspension of a standard virulent strain as

	Mild	Moderate	Severe	Total	Remarks
Mumps	28	14	—	42	Multiple cases and in 14 families (lymphocytesis in 20 cases).
Parotitis	5	1	1	7	
Septic bubo	12	3	0	15	
Traumatic bubo	3	0	0	3	
Abscess	7	7	1	15	
Adenitis (tubercular filarial and venereal)	14	5	—	19	3 ulcer signs, 3 Lymphangitis, 1 Orchitis and 1 Funiculitis
Other acute febrile diseases (meningitis, enteric fever, pneumonia)	1	7	4	12	4 cases died
Others (pleurodynia, otitis media, orchitis, severe anaemia, *etc.*)	10	—	—	10	Otitis media-1, Orchitis-3 Severe anaemia-1
Total	80	37	6	123	

Symptoms and Signs

Symptoms	Mild (80 cases)	Moderate (37 cases)	Severe (6 cases)
Headache	2	0	1
Fever	59	36	6
Pain	50	31	4
Lung symptoms	1	—	—
Delirium	0	0	2
Dry furred tongue	0	0	2
Anxious look	0	0	1
Bloated face	0	1	0
Localisation of Gland Enlargement			
Parotid	30	14	1
Submandibular	7	5	0
Cervical	2	4	1
Axillary	6	5	2
Inguinal	12	11	2
Femoral	7	3	0
Multiple	0	0	3
No enlarged glands	10	7	7

worked out by the auther (Seal 1942). The results showed a good correlation between the smooth colony characteristics, floccular type of agglutination and other serological behaviours on the one hand and 50 per cent mouse protective dose and clinical type of the disease namely, mild, moderate and severe on the other. Both smooth and rough types could be isolated from rat strains, most of them giving floccular or fine floccular (granular) type of agglutination. The virulence of the plague strains isolated from them was rather variable—high, moderate or of comparatively low virulence.

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